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VENTURIA INAEQUALIS (CKE.) WINT. IX. SEARCH FOR MUTANTS RESISTANT TO FUNGICIDES [1]

Curt Leben, D. M. Boone, and G. W. Keitt

SUMMARY

Conidia of wild-type line 365-4 were irradiated with ultraviolet light and plated on media containing 20 times the concentration of sulfur, thiram, antimycin A, or helixin B necessary for the inhibition of this and 3 other wild-type lines. No variants were found resistant to sulfur, thiram, or helixin B. One grew on the medium containing antimycin A, and in subsequent tests was not inhibited by 5000 times the concentration necessary for the inhibition of the wild-type lines. The line resistant to antimycin A, line 3164, was mated *in vitro* with a wild-type line. The ascospores of 44 resultant asci were isolated and cultured separately. On a control medium, 4 spores of each ascus produced slow-growing cultures like 3164, and the other 4 produced larger wild-type cultures. The slow-growing lines of each of the 10 asci tested were not inhibited by a medium containing antimycin A, whereas the 4 wild-type lines were. It is concluded that in 3164 the slow rate of growth and the resistance to antimycin A resulted from a single-gene mutation. Line 3164 and a number of progeny lines containing the mutant gene were tested on apple trees and found to be nonpathogenic. Germinating conidia and sporelings of wild-type lines were more sensitive to the inhibitory action of the antibiotic than were older hyphae.

The possibility that lines of plant pathogenic fungi resistant to fungicides might arise in nature has been long recognized. Yet in the more than 50 years that the old-line copper and sulfur fungicides have been used for controlling plant diseases, no practically significant resistance to them appears to have developed. The problem still exists, however, and whether the same experience with these and other toxicants will prevail remains to be seen, particularly with respect to the newer organic compounds that are now beginning to be used on a wide scale. With certain organisms, e.g. bacteria and insects, there has been a rapid and spectacular rise of forms resistant to the action of certain of the newer organic toxicants. It is thought, therefore, that study of the development of resistance by plant pathogens, particularly to the newer types of compounds, is timely.

The purpose of the work here reported was to explore possibilities of the development of resistance by 1 fungus to 4 fungicides. The fungus *Venturia inaequalis* (Cke.) Wint. is particularly well suited for this purpose (16). In addition to demonstrating a high type of parasitism on the apple plant, the fungus has a sxual stage that enables genetic analysis. Mutant lines altered in pathogenicity, colony morphology, color, or nutritional requirements have been described and studied genetically (2, 15, 16). In the present work, sulfur was used as a representative of the old-line fungicides, thiram (bis(dimethylthiocarbamoyl) disulfide) of the new organic fungicides, and antimycin A and helixin B of the antifungal antibiotics. The antibiotics were included because of current interest in antibiotics for plant disease control and because in the field of medicine the development of resistance by microorganisms to certain of these materials has restricted their usefulness. The approach used in the present work was, in brief, to irradiate conidia of *V. inaequalis* with ultraviolet light and to search among the survivors of the treatment for lines that were capable of growing on amounts of fungicides that inhibited the nonirradiated fungus. A brief account of some of the work has already appeared (19).

MATERIALS AND METHODS.—*Irradiation procedure.*—Wild-type line 365-4 was chosen for irradiation because it incited the lesion reaction on the leaves of many apple varieties and because it had been used in previous mutagenic treatments (2). Conidia were obtained from 2 wick cultures[2] that had been incubated at 16°C for 9 days. Spore suspensions were made in distilled water, pooled (24 ml), and filtered through 2 layers of sterile cheesecloth to remove hyphal fragments. Ten ml of the filtrate, which contained approximately 130,000 conidia per ml, was left untreated for control purposes, and 10 ml was irradiated at 2700–2800 A. Energy input in the system was approximately 7000 ergs and energy absorption approximately 6000 ergs/second. Details of the irradiation apparatus and technique have been given elsewhere (2). Irradiation

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[2] Described by Keitt and Langford (17). The culture fluid contained, per l, extract from 40 g sliced potatoes steamed 0.5 hour in 500 ml water and 5 g of glucose.

time was 22.5 minutes, a period previously demonstrated to permit about 20–30 per cent survival of this line of *V. inaequalis*. Nine ml amounts of irradiated and nonirradiated suspensions were each brought to 180 ml with sterile water for plating on toxicant media. The remainder of each suspension was suitably diluted and plated on a nontoxicant medium to determine the percentage of spores killed by the irradiation treatment.

Media.—The inhibitory effects of the fungicides were noted in tests in which the toxicant was incorporated in basal medium, potato agar[3]. One large lot of basal medium was made at the beginning of the study and stored, and portions were used for each experiment. For each test the basal medium was autoclaved and cooled to about 45°. The toxicant preparation was then added, and the amended medium poured into test tubes or Petri plates.

Stock solutions of thiram (recrystallized from chloroform several times), antimycin A (antimycin A-35 (6)), and helixin B (3.1 units per mg (33)) were made in small volumes of ethanol; a stock sulfur suspension was made by adding flowers of sulfur, unsterilized, to 0.01 per cent (v/v) sterile Tween 80.[4] Stock preparations were added directly to the warm basal medium, or they were diluted with sterile water prior to the addition. Suitable control media demonstrated that the amounts of ethanol or Tween 80 used did not influence the growth of the fungus. The pH of the hardened basal medium was 5.8–6.1 in the different experiments; the addition of the toxicants did not alter the reaction.

Seeding.—For the detection of mutants resistant to the various fungicides, 1 ml of the suspension of irradiated conidia was flooded on the surface of approximately 20 ml of toxicant agar in each Petri dish. There were 40–42 dishes of each of the 4 toxicant media. The companion nonirradiated spore suspension was seeded in the same way to 12 dishes of each toxicant medium.

For the remainder of the experiments, different seeding techniques were used. In agar-streak tests, conidial suspensions containing 80,000–160,000 conidia per ml were streaked with a loop on the surface of media in Petri plates. Usually there were 4 streaks per plate. In tests with single conidia, a spore suspension was flooded on water agar, and after 18–24 hours at 22–24°, germinated spores were lifted individually with a small piece of the agar and transferred to the desired medium. In some tests, mycelium was used; pieces about 1 mm in diameter were taken from the edge of a colony growing on agar. The various types of seedings were made from tube cultures of potato agar or from wick cultures that were incubated 2–3 weeks at 16°.

[3] Per 1: extract from 40 g sliced peeled potatoes steamed 0.5 hour in 500 ml of water; glucose, 5 g; and agar, 17 g.

[4] Surface active agent manufactured by Atlas Powder Co., Wilmington, Delaware.

Mating and pathogenicity tests.—The methods and materials of Keitt and Langford (17) were used to make the *in vitro* mating, to isolate ascospores from individual asci, and to make pathogenicity tests.

RESULTS.—*Inhibition of V. inaequalis by sulfur, thiram, antimycin A, and helixin B.*—Prior to the irradiation experiment, agar-streak tests were made with the 4 toxicants to determine the concentration of each that was necessary for the inhibition of *V. inaequalis*. A series of 2-fold concentrations of each toxicant was made in the basal medium. The range selected for each material was governed by previous work (18, 21). Four wild-type lines of *V. inaequalis* (D5, 365-4, 1031-4, and 1096) were used. For each line at each concentration of a given fungicide there were 4 replicate streaks, each on a different plate. Plates were incubated at 16° for 14 days. At that time the end points (the lowest concentrations of fungicide that prevented macroscopic growth) were determined. For all lines, the end points for sulfur, thiram, antimycin A, and helixin B were 1, 0.12, 0.06, and 1.25 μg/ml, respectively. It may be remarked that these values are for conditions of the test, and that if the conditions are altered (e.g. incubation time or temperature) one may expect different results (20).

Irradiation test.—After the conidia of line 365-4 had been irradiated, suspensions were made as indicated above and flooded on the toxicant media for the detection of variants. For each toxicant about 290,000 spores, of which approximately 27 per cent survived the irradiation treatment, were used. The concentrations of toxicants in μg/ml, in the media were: sulfur, 20; thiram, 2.5; antimycin A, 1.25; and helixin B, 25. These levels were 20 times those necessary for the end point of each toxicant as found in the agar-streak test. The factor of 20 was arbitrarily selected.

After an incubation period of 14 days at 16°, plates seeded with irradiated spores were examined for macroscopic growth. There was none on the media containing thiram, antimycin A, or helixin B. Three small colonies were observed on the sulfur medium. Plates were then incubated 9 additional days at 22–24°. There was still no growth on the thiram or helixin B media, and the 3 colonies on the sulfur medium had become larger. On the antimycin A medium a sparse growth, not easily detected with the naked eye, appeared on each plate; in addition there was 1 large colony, which was easily seen and obviously different from the remainder of the growth. The nonirradiated control spores (approximately 86,000 spores on each medium) did not grow on the sulfur, thiram, or helixin B media; on the antimycin A medium a sparse growth, of a type similar to that arising from irradiated spores, was observed. Stock single-spore cultures were made from the 3 colonies on the sulfur medium and from the large colony on the antimycin A medium.

It is noteworthy that these variant lines were derived from irradiated spores and that none was obtained from nonirradiated spores. It is not possible to attribute their production to the irradiation treatment,

however, since the total number of variants detected was small.

Effect of sulfur on lines from the sulfur medium.—These 3 lines were again tested on sulfur medium (20 μg/ml) in 2 agar-streak tests made at different times. There was no growth of any line. It appeared, therefore, that these were not mutants, and for this reason they were not investigated further. It would be of interest to determine what was responsible for their growth following irradiation.

Effect of antimycin A on the line from the antimycin A medium.—This isolate, designated 3164, was also tested in 2 agar-streak tests made at different times. There was 1.25 μg/ml of antimycin A in the medium. In each test, 3164 grew as well on the antimycin A medium as it did on the basal medium, although growth on both media was slower than that of 365-4 on the basal medium (Fig. 1).

An additional agar-streak test was made to note the effects of greatly increased amounts of antimycin A on 3164. A series of 4-fold concentrations of antimycin A ranging from 1.25 to 320 μg/ml of basal medium was made. Media were seeded with spores of both 3164 and 365-4, using 2 streaks of each line on each of 4 plates at each concentration. After 14 days at 16° there was no difference in the amount of growth of 3164 on the control medium and on any medium containing antimycin A. The results were the same after an additional 9 days at 22–24°. Growth of 365-4 on all antimycin A media was barely discernible at 14 days, and after the 9 additional days at the higher temperature growth had not increased materially. Line 365-4 grew vigorously on the control medium.

These experiments demonstrated that antimycin A had little or no effect on the growth of 3164. Even with a medium containing 320 μg/ml, some 5000 times the end point for 365-4 and 25 times the amount in the medium on which irradiated spores were placed, 3164 grew as well as it did on the control medium. With wild-type 365-4 there was a nongradient type of inhibition: a small amount of the antibiotic eliminated nearly all growth of this line, but contrary to usual results with fungicides, increasing amounts did not produce further inhibition. This experience with 365-4 parallels that of Lockwood et al (23), who found a nongradient inhibition of a variety of fungi over a wide concentration range by the closely related antibiotic, antimycin A-102. Nongradient inhibition has

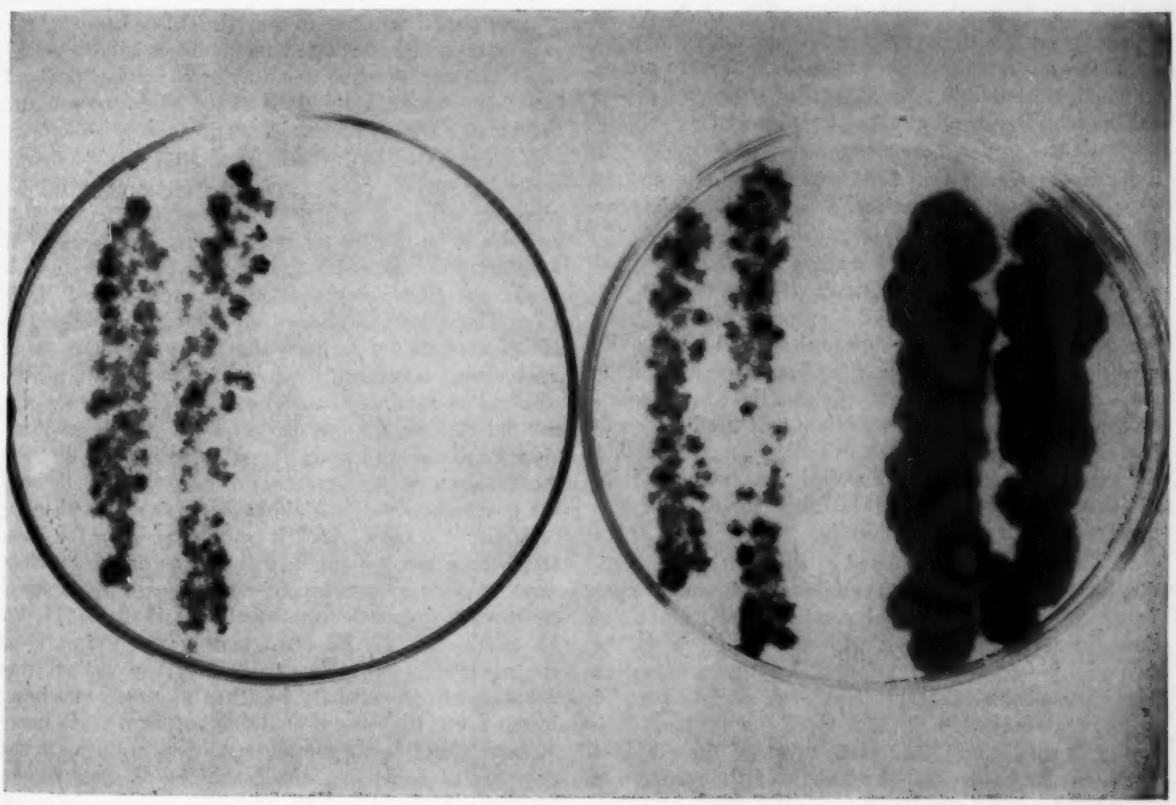

FIG. 1. Growth of the antimycin A resistant line and of a wild-type line on basal medium with and without antimycin A. Left Petri plate: antimycin A medium. Right plate: basal medium. In each plate the 2 streaks on the left are the antimycin A resistant line, and 2 streaks on the right are the wild-type line from which the resistant variant was derived. Note the inhibition of the wild-type line on the antimycin A medium and absence of inhibition of the resistant line on that medium.

been described also for mutants of Neurospora resistant to sulphanilamide (7) and to canavanine (14).

Studies on the progeny of 3164.—Line 3164 was mated with a wild-type isolate of the opposite mating type, line 1096. The 8 ascospores from each of 44 asci from the mating were isolated in serial order and grown on basal medium. Four ascospores from each ascus produced large wild-type colonies and the other 4 slow-growing colonies like 3164. There was 43.2 per cent second division segregation.[5] Thus, the gene governing small colony size segregated in a 1:1 fashion, and its distance (uncorrected) from the centromere was approximately 21.6 morgans.

By means of mycelium pieces, transfers from the 8 ascosporic cultures of each of 10 asci were made to the basal medium or to the basal medium containing 80 μg/ml of antimycin A. After 4 weeks at 16° on the basal medium, the growth of the progeny lines was of the same type observed at the time of the original isolation (4 small colonies and 4 larger wild-type colonies). On the antimycin medium the small lines were not inhibited. On the other hand, growth of wild-type lines was retarded but approached that of the small lines on that medium. This significant growth of the wild-type lines on the antimycin A medium was not expected and was the subject of further studies using different seeding methods (see following section). In an additional test the 8 lines from 1 ascus were grown on medium containing 1.2 instead of 80 μg/ml of the antibiotic. The results were the same at both concentrations.

It was concluded that 3164 carried a single mutant gene for resistance to antimycin A and that this gene also governed colony size.

Influence of the type of seeding on the growth of wild-type lines on the antimycin A medium.—It has been noted that when conidial suspensions were used for seeding wild-type lines on antimycin A media, the resulting growth was meager and usually difficult to see. In contrast, the growth of these lines approached that of the mutant when mycelium was used for seeding these media. These observations suggested that the method of seeding influenced the amount of inhibition of the wild-type lines. A series of tests was made to examine this hypothesis.

In 3 experiments completed at different times, conidia were streaked on unsupplemented water agar (agar, 17 g/1) or on basal medium, and on each of these media supplemented with the antibiotic at the rate of 1.2 μg/ml. It was found that the germination of spores of the wild-type lines after 48 hours at 16 or 24° was limited to from 1 to 16 per cent on antimycin A media and that germ tubes of these spores were usually about half the length of the same lines on control media. In contrast, over 90 per cent of the spores of line 3164 germinated on the antimycin A or control media, and the germ tube length was not reduced by the antibiotic.

When conidia of wild-type lines or of the mutant were germinated on water agar and the sporelings transferred singly to basal medium, there was vigorous growth at 16 or 24°. If, however, wild-type sporelings were put on the basal medium containing antimycin A, not all of them formed a mycelium, there being fewer mycelia at 24 than at 16°. In contrast, at either temperature sporelings of the mutant always grew on the antimycin medium.

Mycelia were always produced at either temperature when mycelium was used to seed either the mutant or the wild-type lines on the antimycin A medium.

It is concluded that a small percentage of conidia or conidial sporelings of wild-type lines of *V. inaequalis* is able to grow in a limited fashion in the presence of antimycin A. Once past the sporeling stage all of the wild-type lines that were tested were able to grow at a much reduced rate in the presence of the antibiotic. Another finding, not dealt with in detail here, was incidentally discovered: 3164 grew better on the basal medium at 24 than at 16°. This is in contrast to 365-4, which grew at about the same rate at both temperatures.

Pathogenicity tests.—The antimycin A resistant mutant and a number of progeny lines carrying the resistance gene were tested for pathogenicity to potted McIntosh apple trees. No line was pathogenic in 3 tests; however, 365-4 and all of the wild-type progeny lines tested were pathogenic.

Discussion.—In the present studies we have described a variant of *V. inaequalis* resistant to the inhibitory action of the antibiotic antimycin A. This appears to be the second report of the development of a resistant line of a plant pathogenic fungus to an antibiotic, the first being that of Christensen and Davis (5). These workers described pathogenic variants of *Helminthosporium sativum* that were resistant to an unidentified substance produced in the culture filtrate of *Bacillus mesentericus*. The order of resistance was not determined. In the present work the order of resistance of the antimycin A variant was such that the mutant grew in the presence of more than 5000 times the concentration of antibiotic that was required to inhibit the line from which it was derived. In the literature there are a number of instances of the development *in vitro* of resistance by plant pathogenic fungi to toxicants other than antibiotics (1, 4, 9, 10, 11, 12, 13, 22, 24, 25, 27, 34, 38). In the studies just cited the order of resistance attained by the variant was not stated or was usually less than 10 times, and in no instance was the variant analyzed genetically. In many of these experiments the pathogen was cultured in the presence of increasing concentrations of the toxicant. It seems likely, therefore, that the resistant lines obtained were the result of multi-step mutations, assuming the *modus operandi* to have been genetic in the Mendelian sense. With the antimycin A mutant, it was possible to show that the character for resistance was

[5] Details on types of segregation will appear elsewhere.

inherited in a Mendelian fashion, and that it was produced by a first-step mutation. In the related nonpathogenic fungus Neurospora, Mendelian genetic control for resistance to sulphanilamide (7) and to canavanine (14, 37) has been described.

Taylor (36) recently has reported results that he interpreted as indicating the presence of lines of *Physalospora obtusa* resistant to Bordeaux mixture in certain orchards that had been sprayed with that fungicide for a number of years. If consistent and significant resistance of lines of *P. obtusa* to Bordeaux mixture can be demonstrated, a study of the degree and the origin of this resistance would be of much interest.

To be significant in crop production it is necessary for a resistant variant to be pathogenic, which was not the case with the *V. inaequalis* mutant that was obtained. Should a resistant mutant be pathogenic, there are other important factors that might govern its ultimate survival and importance. Among these are the rate and manner of spread of the particular pathogen, the proximity of plants of susceptible varieties, the response of the mutant to the environment, and its resistance to other fungicides in situations in which more than 1 fungicide is employed.

Further study of the development of resistance to toxicants by plant pathogens would be desirable and might aid in the development of new fungicides and bactericides for use on plants. Recent experiments of this sort with bacteria are those of English and VanHalsema (8). Using selective techniques, they found that isolates of *Erwinia amylovora* and *Xanthomonas vesicatoria* became resistant to streptomycin and a combination of streptomycin and oxytetracycline, the degree of resistance attained being less with the combination. In view of the possible widespread use of antibiotics for the control of plant diseases incited by bacteria and of the capacity of certain bacteria for becoming resistant to 1 or to a group of antibiotics (e.g. 26, 35), the findings of English and VanHalsema may be portentous. Their report, however, made no mention of tests for pathogenicity with their resistant cultures.

There are several implications in the present work with respect to the mode of action of antimycin A. In the system concerned with the transfer of electrons from dehydrogenases to cytochrome *c*, this antibiotic has been shown to be a powerful pseudoirreversible inhibitor that acts on a component between cytochrome *b* on the one hand and cytochrome *c* on the other (3, 29, 30, 31). Our data suggest that, since the antibiotic has little or no inhibitory action on the resistant mutant, the conventional cytochrome system is not essential in this line. Terminal oxidation may take place via some alternative pathway, possibly such as has been suggested (28, 32). If this is so, and since the mutant was found to be nonpathogenic, the further inference can be made that the operation of the cytochrome system is essential for pathogenicity in this fungus. A third inference is that, since the antibiotic was not completely inhibitory to wild-type lines, the major portion of the terminal oxidation of these lines proceeds via the cytochrome system, and the small remainder goes by way of an antimycin A insensitive pathway. Such a hypothesis would offer an explanation for the existence of the nongradient type of inhibition of these lines. These inferences need to be studied further for verification or refutation.

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THE INTERRELATIONSHIP OF POTATO AND TOMATO RACES OF PHYTOPHTHORA INFESTANS [1]

J. B. Wilson and M. E. Gallegly

SUMMARY

The reactions of 29 potato isolates of *Phytophthora infestans*, representing 10 different potato races, were studied on tomato differential hosts. Similarly, 16 tomato isolates, representing 3 different tomato races, were studied on potato differential hosts. The potato isolates fell into 4 groups: Group I, weakly pathogenic to nonpathogenic to all tomato varieties; Group II, pathogenic to the recessive varieties Marglobe, Rutgers, and Bonny Best but nonpathogenic to the multiple-gene varieties Wisconsin "55" and 19 and dominant-gene accessions 36 and 106; Group III, pathogenic to the recessive and multiple-gene varieties but not to the dominant-gene accessions; and Group IV, pathogenic to all the tomato hosts. Isolates of Group II and III differed only in degree of virulence. There was no correlation between potato-race characteristics and reaction on tomato hosts. One isolate of a given potato race may react as one tomato race while another may react as a different tomato race. A similar relationship exists in regard to tomato races on potato. The tomato isolates reacted as potato races 0, 3, or 4. The tomato genes are different from any now known in potato; the race characteristics of a given isolate on potato should be considered independently from its characteristics on tomato.

Physiologic races of *Phytophthora infestans* (Mont.) dBy. were recognized by Giddings and Berg (6) in 1919, when they found that an isolate taken from potato was pathogenic only to potato, whereas an isolate from tomato was pathogenic to both potato and tomato. This work is the basis for the common usage of the terms "potato race" and "tomato race". Mills (9) reported that the "potato race" could be changed to the "tomato race" by successively passing the former through tomato foliage.

More recently, 16 physiologic races were described within Berg's (1) original "potato race". Black (2), in Scotland, and Mastenbroek (7, 8), in the Netherlands, each found 4 independent dominant genes for resistance. Similarly, Mills and Peterson (10) in the United States found 3 genes for resistance. Each worker had his own particular set of differential hosts and method of designating races. The joint work of Black, Mastenbroek, Mills, and Peterson (3) and the work of Pristou and Gallegly (11) show that these potato races and genes for resistance, as individually described, agree closely and fall into a definite pattern. As a result of the joint work (3), an international scheme for designating potato races of *P. infestans* and genes for resistance to these races was proposed.

Recently, Gallegly (4) found that physiologic races of the fungus occur within Berg's original "tomato race". Further work (5) showed that 1 type of resistance to the common tomato race was controlled by a single dominant gene (major gene) and that another type was controlled by multiple genes.

Since potato physiologic races of *P. infestans* were described by 1 group of workers and tomato physiologic races were described by another, it is desirable to determine if the genes for resistance found in tomato are identical to or different from those occurring in potato and if there is a relationship between potato-race and tomato-race characteristics. This paper reports the results of such a study.

METHODS AND MATERIALS.—Tomato hosts used were varieties Marglobe, Rutgers, and Bonny Best, which are susceptible; West Virginia accession 19 and Wisconsin "55", which have multiple-gene resistance; and accessions 36 and 106, which have dominant-gene resistance to the common tomato race. The potato differential hosts and their genotypes are listed in the tables of experimental results. Essex, 3RC-8, DXD-3, 3XE-1, 3WM-19, TI-5, and 3XX-1 were received from W. R. Mills, Pennsylvania State University, University Park, Pennsylvania; Pentland Ace, 1682c(1), and 1488b(1) were from W. Black, Scottish Plant Breeding Station, Edinburgh, Scotland; and 4739-58 was from C. Mastenbroek, Plant Breeding Station Centraal Bureau, Hoofddrop, the Netherlands. Potato hosts with all the possible combinations of the 4 genes were not available; those used were of the single- and the double-gene combinations.

Plants were grown in greenhouse soil in 3- and 4-in. pots. Tomato plants, 10 to 15 per pot, were 3–5 weeks old when inoculated. Potato plants, 1 per pot, were 4–6 weeks old and ranged in height from 4–12 in. when inoculated; 3 plants of each potato host were used.

Foreign isolates of potato races were obtained from W. Black and C. Mastenbroek; United States isolates were obtained from W. R. Mills and from L. Cash, Plant Industry Station, Beltsville, Maryland; in addition, several local West Virginia isolates were used. Tomato isolates were either from West Virginia or were sent in from other places for identification. Race characteristics and sources of the isolates are given in

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the tables of experimental results.

The isolates, cultured on lima bean agar (frozen lima beans ground for 10 minutes with Waring Blendor, 250 g; agar, 15 g; distilled water to make 1000 ml), were 10–14 days old when used for inoculations. Sporangium-zoospore inoculum, prepared by incubating sporangial suspensions for about 2 hours to induce zoospore liberation, was sprayed on the plants which were then placed in a room maintained at 20°C and 100 per cent relative humidity. After 24 hours the plants were removed to a greenhouse maintained at approximately 20°C.

The following disease index was used in rating the plants 7 days after inoculation: 1) Immune, no evidence of infection; 2) Highly resistant, plants show specks and small lesions that do not spread; 3) Resistant, lesions larger but appear arrested, stem lesions do not girdle plant; 4) Susceptible, lesions of typical spreading type but plant not dead; 5) Very susceptible, plants dead or dying. Plants with average indices 3.5 or above were considered susceptible; those with readings below 3.5 were considered resistant.

EXPERIMENTAL RESULTS.—*Reaction of tomato differential hosts inoculated with isolates of potato races.*—Potato isolates fell into the following 4 groups when their pathogenicity to tomato differential hosts was compared (Table 1):

Group I: Pennsylvania A, B, and D; Scotland B^2, C, G, and H; the Netherlands N8; Beltsville GM, 89, 182, 201, 256, and 583; West Virginia WV-1.

Group II: Pennsylvania BD; Scotland F; the Netherlands N4 and N6; Beltsville 544; West Virginia WV-4 and WV-5.

Group III: Pennsylvania C and BC; Scotland B^1 and D; the Netherlands N7 and N9.

Group IV: the Netherlands N1.

Isolates in Group I are weakly pathogenic on all the tomato hosts and correspond to Berg's (1) original "potato race"; those in Group II are pathogenic to the recessive varieties Marglobe, Rutgers, and Bonny Best, but are weakly pathogenic to nonpathogenic to the multiple-gene resistant varieties Wisconsin "55" and West Virginia accession 19 and to the dominant-gene resistant accessions 36 and 106. Group II isolates correspond to Gallegly's original tomato race 1 (4). Group III isolates are pathogenic to the recessive and multiple-gene hosts but not to the dominant-gene

TABLE 1.—*Disease index readings of different tomato hosts when inoculated with potato isolates of P. infestans; indices are averages of 2 or 3 experiments, with 1 indicating immunity and 5 high susceptibility*

International potato race	Source and Isolate	Tomato differential hosts						
		Marglobe	Rutgers	Bonny Best	Wis."55"	19	36	106
	Pennsylvania							
0	A	2.8	2.5	2.5	2.5	2.0	1.3	1.0
4	B	3.0	3.0	2.3	2.7	2.0	1.0	1.7
2	C	4.0	4.5	3.5	4.0	3.5	2.0	2.0
1	D	2.5	3.0	2.0	2.0	1.5	1.5	1.5
2,4	BC	4.5	4.3	4.3	4.3	4.0	2.3	2.3
1,4	BD	3.7	3.7	3.7	3.3	3.0	2.3	2.0
	Scotland							
1	B^1	4.0	4.0	4.0	4.0	4.0	2.0	2.0
1,4	B^2	2.0	2.0	2.5	1.5	2.0	2.0	1.0
2,4	C	2.0	2.0	2.0	1.0	1.0	1.0	1.0
4	D	4.0	4.5	4.0	4.0	4.0	2.5	2.0
1,3,4	F	4.0	4.5	3.5	2.5	2.5	2.0	1.5
1,2	G	3.0	3.0	3.0	2.0	2.0	2.0	1.5
2	H	3.0	3.0	3.0	3.0	2.0	2.0	1.0
	The Netherlands							
0	N1[a]	5.0	5.0	5.0	5.0	5.0	5.0	5.0
4	N4[a]	4.0	4.0	4.0	3.5	3.0	2.0	2.3
2,4	N6	4.0	4.7	4.0	3.3	3.3	2.0	2.0
1,4	N7	4.0	4.0	4.0	4.0	4.5	2.0	2.0
1,2,4	N8	2.7	2.7	2.7	1.7	1.3	1.7	1.2
2,3,4	N9	4.0	4.0	4.0	4.0	4.0	2.0	2.0
	Beltsville, Maryland							
4	GM	2.5	3.0	3.0	2.5	2.0	1.5	1.5
1	89	2.0	2.0	2.0	2.0	1.0	1.0	1.0
1,4	182	3.0	3.0	3.0	2.0	1.0	1.0	1.0
0	201	3.0	3.0	3.0	2.0	2.0	2.0	1.5
1	256	2.0	1.5	1.5	1.5	1.0	1.5	1.0
1,4	544	4.0	4.0	3.5	3.0	2.5	1.5	1.5
1,4	583	2.0	2.5	2.5	2.0	1.0	1.0	1.5
	West Virginia							
0	WV-1	3.0	3.0	2.0	2.0	2.0	1.0	1.0
1,4	WV-4	3.5	3.5	3.0	3.0	2.0	2.0	2.0
1,4	WV-5	3.5	3.5	3.5	3.7	2.5	2.0	2.0

[a] The N1 isolate was received as N2 but when tested was found to be potato race 0, and its number was changed to correspond to the proper Netherland race; likewise, N4 was received as N1.

resistant accessions. The isolates in Group III correspond to Gallegly's original tomato race 2. The isolate in Group IV is highly pathogenic to all tomato hosts. It corresponds to Gallegly's original tomato race 3.

Fifteen of the 29 potato isolates fall into Berg's original "potato race" group, whereas 14 fall into Berg's original "tomato race" group. Of the latter group (Group II), 7 isolates are pathogenic only to the recessive varieties, 6 (Group III) are pathogenic to both the recessive and multiple-gene hosts, and 1 (N1) is pathogenic to all tomato hosts used.

The isolates in Group III (pathogenic to the multiple-gene hosts) are more pathogenic to the recessive varieties than are the isolates of Group II (nonpathogenic to the multiple-gene hosts). This indicates that the main difference between the isolates of Group II and Group III is in the degree of virulence.

There is no correlation between the potato-race characteristic and reaction on the tomato hosts. For instance, the Scotland potato race 2.4 was placed in Group I; the Netherlands race 2,4 in Group II; and the Pennsylvania race 2,4 in Group III. Similarly, the Pennsylvania potato race 0 and the West Virginia potato race 0 were placed in Group I, whereas the Netherlands race 0 was placed in Group IV.

Reaction of potato differential hosts inoculated with tomato races.—Isolates of the tomato races were identified as potato races 0, 3, or 4 (Table 2). Isolates F2, F3, F4, M-7, 6T-2, and R-1 reacted as potato race 0; isolates 6T, 6T-1, 6T-3, 6T-4, and NH-1 were identified as potato race 4; and isolate 2T was potato race 3. Isolate F1 reacted as would be expected for a mixture of potato races 0 and 3; only 1 of the 3 plants of the R_3 genotype was susceptible, whereas all of the recessive plants were susceptible. Similarly, isolate M-13 reacted as a mixture of races 0 and 4. Isolates M-6 and R-2 were not pathogenic to any of the potato hosts; a recheck of these isolates on tomato showed that they had lost also the ability to infect the tomato hosts. There is no correlation between the tomato-race and potato-race characteristics. For instance, 3 isolates of Gallegly's original tomato race 1 reacted as potato race 0, whereas 5 isolates reacted as potato race 4. Furthermore, isolate 6T and 3 of the 4 single-zoospore cultures from it reacted as potato race 4 and one as potato race 0. This indicates that 6T is a mixture of potato races 0 and 4; it was not identified as a mixture when a heavy zoospore-sporangium suspension was used in the inoculation. Similarly, of the isolates of the original tomato race 3 of Gallegly, 3 isolates reacted as potato race 0, one as potato race 3, one as a mixture of potato race 0 and 3, and one as a mixture of potato races 0 and 4.

Potato race 3, previously not reported as occurring in the United States, was present in isolates 2T and F1. Isolate 2T was obtained in 1950 from an infected tomato plant growing at Huttonsville, West Virginia, and isolate F1 was obtained in 1952 from a tomato

TABLE 2.—*Disease index readings of differential potato hosts when inoculated with tomato isolates of P. infestans; indices are averages of 2 or 3 inoculations, with 1 indicating immunity and 5 high susceptibility*

		Differential host and international genotype											
Tomato race	Isolate and source[a]	Cobbler rr	Essex R_1	3RC-8 R_2	Pentland Ace R_3	DXD-3 R_4	3XE-1 R_1R_2	4739-58 R_1R_3	3WM-19 R_1R_4	1682c (1) R_2R_3	TI-5 R_2R_4	1488b (1) R_3R_4	3XX-1 $R_1R_2R_4$
	Florida												
3	F1	5.0	2.0	2.7	3.3[b]	2.3	2.0	1.0	2.0	1.3	1.7	1.0	2.0
3	F2	5.0	2.0	2.0	1.0	2.7	1.7	1.0	2.3	1.0	1.7	1.0	1.3
3	F3	5.0	1.7	1.7	1.7	1.0	2.3	2.0	2.7	1.5	2.3	2.7	2.0
3	F4	4.0	2.0	1.7	1.3	1.3	1.3	1.0	1.3	1.5	1.0	2.0	1.7
	Beltsville, Md.												
2	M-6	1.7	1.0	2.7	1.0	1.3	1.0	1.7	1.7	1.0	2.0	1.0	1.3
1	M-7	4.0	1.3	1.7	1.0	1.7	2.0	2.0	2.0	1.0	1.7	1.0	1.0
3	M-13	4.0	1.5	1.7	2.0	3.3[b]	1.3	1.7	2.0	1.0	1.0	1.5	1.7
	West Virginia												
3	2T	4.7	1.0	2.0	4.5	1.7	1.0	2.7	1.3	1.0	1.3	1.0	2.3
1	6T	5.0	3.0	2.7	3.0	4.7	1.0	1.3	2.0	1.0	2.3	2.0	2.0
1	6T-1[c]	4.5	2.0	2.0	1.0	3.7	2.3	1.3		1.5	1.3	2.0	1.3
1	6T-2	4.0	2.7	2.3	2.3	2.5	2.3	2.0		2.0	2.0	2.3	1.7
1	6T-3	4.0	1.0	2.0	1.7	3.7	1.3	2.0	1.5	2.0	1.3	2.0	1.3
1	6T-4	4.3	2.3	2.0	2.0	3.7	2.0	2.0	2.0	2.5	1.7	2.7	1.7
	Canada												
1	R-1	3.7	1.3	1.5	1.0	1.3	1.3	1.0	1.3	2.7	2.0	1.0	1.5
1	R-2	2.0	1.0	1.0	1.0	1.3	1.0	1.0	1.0	1.0	1.0	1.0	1.3
	New Hampshire												
1	NH-1	5.0	1.7	2.3	1.0	5.0	1.0	1.7	2.0	1.0	2.7	2.0	1.0

[a] Florida isolates were from R. A. Conover, Sub-Tropical Experiment Station, Homestead, Florida; Beltsville isolates from Paul R. Miller, Plant Industry Station, Beltsville, Maryland; Canadian isolates from K. M. Graham, Department of Agriculture, Ottawa, Ontario; and the New Hampshire isolate was from A. E. Rich, University of New Hampshire, Durham, New Hampshire.

[b] One of 3 plants showed a susceptible 4 reaction.

[c] 6T-1, -2, -3, and -4 are single-zoospore cultures from isolate 6T.

plant growing at Homestead, Florida.

DISCUSSION.—There is no definite relationship between the physiologic potato-race and tomato-race characteristics of *Phytophthora infestans*. The dominant genes for resistance in tomato accessions 36 and 106 are distinct from any of the 4 dominant genes now known in potato. Two potato isolates reacting as the same potato race may react entirely differently on tomato. Likewise, different isolates of the same tomato race may react as entirely different potato races on potato differential hosts.

Thus, at present the potato-race characteristic of a given isolate should be considered independently of its tomato-race characteristic. Since there is little or no possibility of incorporating the tomato genes into potato varieties or *vice versa*, it seems desirable to use a separate scheme to designate the tomato races and genes for resistance to them.

Potato isolates pathogenic to multiple-gene tomato varieties were more pathogenic on varieties without such genes (Marglobe, Rutgers, and Bonny Best) than were isolates which would not attack the multiple-gene varieties. This indicates that perhaps the main difference between Gallegly's original tomato races 1 and 2 is in degree of virulence. It is possible that the more virulent isolates of his race 1 are strongly pathogenic on the varieties possessing multiple-gene resistance whereas those isolates with a lower degree of virulence are weakly pathogenic to these varieties. The fact that the M-6 isolate, originally described by Gallegly (4) as tomato race 2, is weakly pathogenic to both the potato and tomato hosts indicates that an isolate may lose virulence in culture and react differently in later tests.

It is suggested that Gallegly's original tomato races 1 and 2 be considered as 2 isolates of the same race differing only in virulence. Recently, Gallegly and Marvel (5) suggested a system of designating tomato physiologic races and genes for resistance to these races which parallels the one used for designating potato races and genes. The original races 1 and 2 were designated as tomato race 0 whereas the original race 3 was labeled tomato race 1. Because of the possibility of confusion, it is further suggested that the potato gene and race numbers be prefixed with a "P" and tomato numbers with a "T."

The results presented herein further confirm the early works of Giddings and Berg (6), Berg (1), and Mills (9) which showed the existence of "potato" and "tomato" races of *Phytophthora infestans*. In addition, the results confirm that there are physiologic races not only within the original "potato race" but also within the original "tomato race."

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EVIDENCE OF INJURY TO DECIDUOUS FRUIT TREES BY AN ECTOPARASITIC NEMATODE (XIPHINEMA SP.) AND A PROMISING CONTROL MEASURE [1]

Robert E. Adams[2]

SUMMARY

Young peach and apple trees growing in soil treated with benzene hexachloride showed increased growth apparently due to the demonstrated control of *Xiphinema* sp., an ectoparasitic nematode. Injury to roots of older apple trees by this nematode may cause the condition known locally as "pout". One season's treatment with benzene hexachloride improved the appearance of such apple trees, reduced the nematode population in the soil, and caused no abnormal flavor in the fruit.

In 1948, Gould and associates initiated studies at the West Virginia University Experimental farm at Kearnysville to determine the influence of an accumulation of various spray materials in the soil on fruit tree growth. Gould and Hamstead (3) in 1951 reported that increased growth of peach and apple trees resulted from soil applications of spray-grade benzene hexachloride (BHC) containing 10 per cent gamma isomer. Some of the response of the apple trees to this treatment was attributed to the control of wooly apple aphid, *Eriosoma lanigerum* Haus. The authors indicated, however, that the increase in tree vigor resulting from the treatment could not be explained entirely by the control of wooly apple aphid. No conclusive evidence of the nature of the contributing factor was obtained. The peach trees were not infested by the wooly aphid.

The influence on soil nitrification of some of the materials tested in the above experiment was studied by Wilson (7), who found that BHC had little or no effect upon the nitrification process up to a rate of 400 lb. per acre. When the dosage rate of BHC was increased to 2000 lb. per acre an inhibitory effect upon nitrification was found. Wilson showed that the increased growth was not correlated with increased nitrification.

Several statements in the literature indicate that the dagger nematodes (*Xiphinema* spp.) may be parasitic on woody plants (1, 5, 6). It was not until 1954 that experimental proof of the feeding of *Xiphinema* spp. on plant roots was presented (4).

This paper reports briefly evidence obtained at the West Virginia Agricultural Experiment Station that nematodes of a species of *Xiphinema* are parasitic on apple and peach tree roots and that they are the probable cause of a disorder of apple trees known locally as "pout".

EVIDENCE OBTAINED FROM PEACH TREES.—In 1949, another peach tree planting was made by Gould, as a continuation of the previous study (3). Some of the treatments in this experiment were as follows: spray-grade BHC (10 per cent gamma isomer) applied at the rate of 30 oz./100 sq. ft., 30 oz./100 sq. ft. 2 years in succession, 36 oz./100 sq. ft., 90 oz./100 sq. ft.[3], and a control consisting of no treatment other than the usual cultural practices. The BHC was suspended in water and applied with a sprinkling can to the experimental area. The experimental planting was removed during the winter of 1953-54.

Nematodes found.—In the search for the cause of the increased growth of peach trees following soil applications of BHC, samples of soil taken from around roots of the treated and untreated peach trees were examined for the presence of plant parasitic nematodes. At the time the trees were pulled, 1-lb composite

[3] This 90-oz. treatment was applied to only 10 sq. ft. about each tree (9 oz./10 sq. ft.).

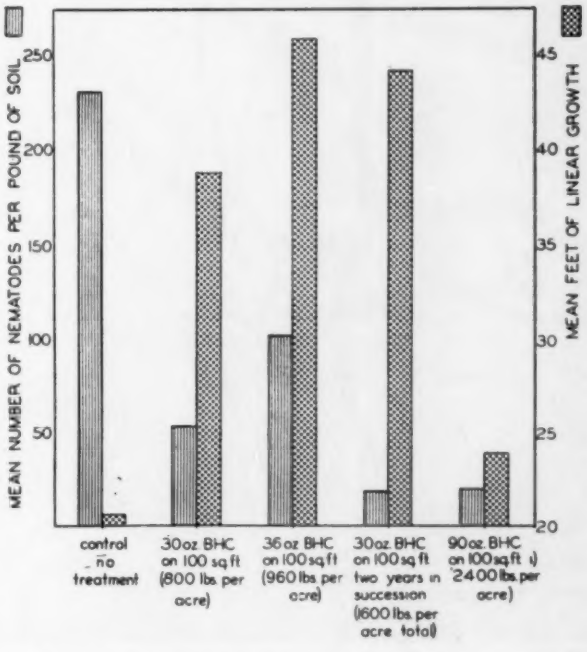
MEAN NUMBER OF NEMATODES PER POUND OF SOIL
250
200
150
100
50
45
40
35
30
25
20
MEAN FEET OF LINEAR GROWTH
control – no treatment
30 oz BHC on 100 sq ft (800 lbs per acre)
36 oz BHC on 100 sq ft (960 lbs per acre)
30 oz BHC on 100 sq ft two years in succession (1600 lbs per acre total)
90 oz BHC on 100 sq ft [3] (2400 lbs per acre)

FIG. 1. Linear growth of peach trees and nematode populations in soil plots treated with BHC.

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soil samples were taken in a uniform manner in 3 replications from the area that each tree occupied. The soil samples were taken to the laboratory and processed by the Baermann funnel technique (2). At this time it was noted that the odor of BHC could be detected in the soil samples from the BHC treatments.

The collected nematodes were counted by means of a dissecting microscope. A representative sample from each collection of nematodes was identified by Mr. A. L. Taylor, Division of Nematology, U. S. Department of Agriculture, Beltsville, Maryland. Over 90 per cent of the nematodes in the populations were *Xiphinema* sp. and closely resembled *X. americanum*. No other plant parasitic nematodes were found in the soil, and no evidence of attack by root-knot or other endo-parasitic nematodes was observed on the roots.

Response of peach trees related to nematode populations.—The total linear feet of growth that each peach tree made was measured by Mr. Gould in April of 1952. During the course of the experiment, the trees in the plots receiving the 3 intermediate BHC treatments (30 oz. in 1 application, 30 oz. applied 2 years, and 36 oz. in 1 application per 100 sq. ft.) made twice as much linear growth as the control trees or those that received the highest dosage of BHC (90 oz./100 sq. ft.). The nematode population was found to be much lower in the BHC-treated plots than in the controls (Fig. 1). There was a negative correlation between tree growth and nematode population (significant at the 1 per cent level) for all the treatments with the exception of the high dosage rate of BHC. At this high dosage the trees showed no increase in growth over the controls. These results suggest that the nematodes found were a limiting factor in the growth of the peach trees in this experiment, except for the high BHC treatment where the concentration of BHC was probably toxic. The typical appearance of peach roots from the untreated and BHC-treated trees is shown in Figure 2. The most striking difference is the almost complete lack of feeder roots on the

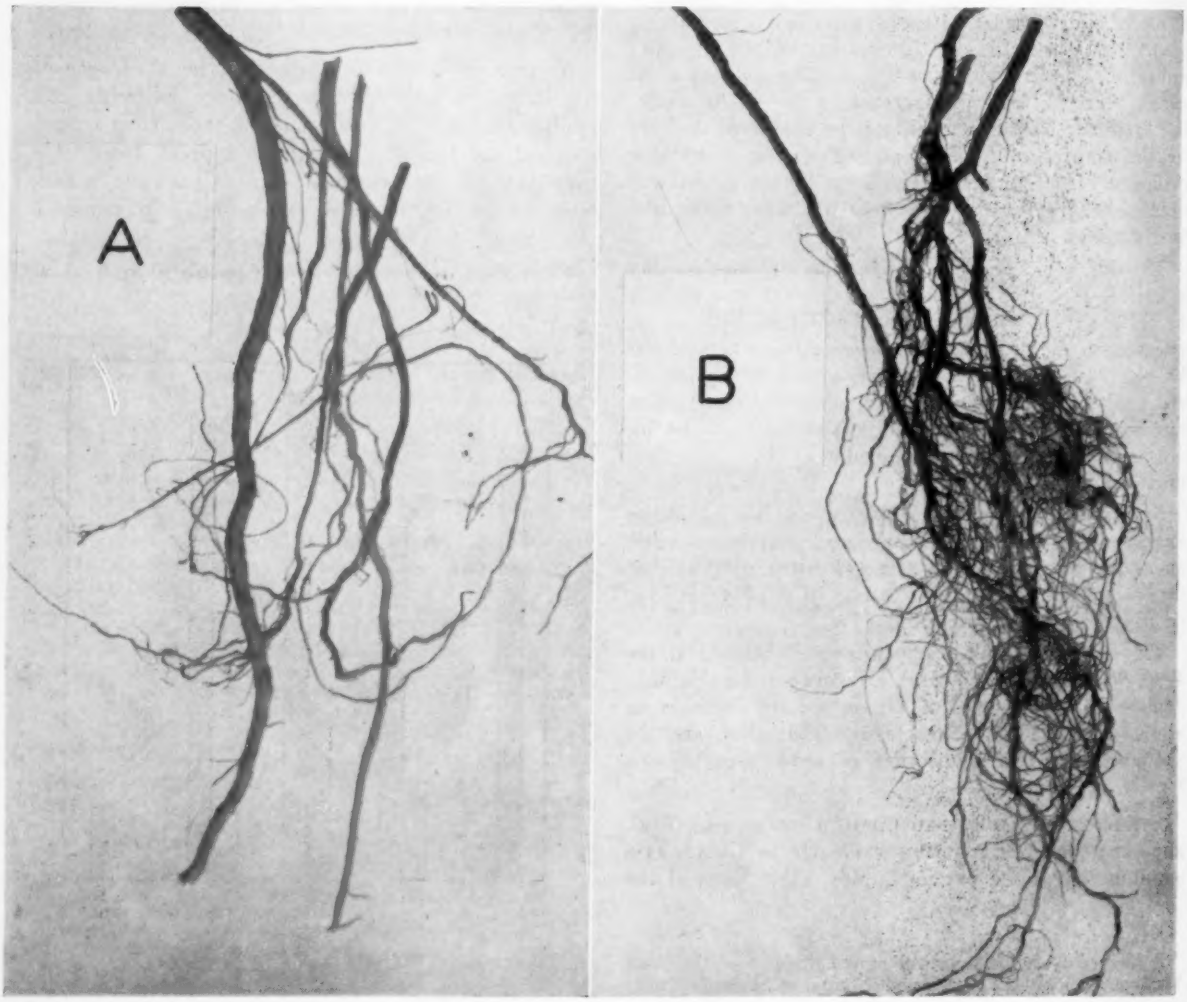

FIG. 2. A) Root specimens from untreated peach trees. B) Root specimens from peach trees grown in soil treated with 30 oz. of BHC on 100 sq. ft. of soil surface.

specimen from the untreated control trees and the relative abundance of them on the specimen from the BHC-treated trees. There were no other symptoms shown by the peach trees in this experiment other than the relatively slow growth and the sparse root system of the control trees.

EVIDENCE OBTAINED FROM APPLE TREES.—In 1939, a 70-acre apple orchard in the vicinity of Romney, West Virginia, was replanted on the site of what had been one of the best producing apple orchards in the area. The replant trees made satisfactory terminal growth for the first 4 or 5 years, then the trees started to decline and, as the grower described it, "went into a pout." "Pout" is an indigenous and perhaps fitting term for this disorder. It is applied to fruit trees that fail to make satisfactory growth and fail completely to respond to excessive fertilization. These trees may exhibit die-back, and their foliage is off-color (yellowish green instead of dark green). Terminal growth ranges from ¼ to 3 in. with an occasional terminal making greater growth. The trees are about 9–12 ft. tall and appear generally unthrifty. The yield is so low that continued operation is not economically sound and removal of the trees is contemplated.

Nematodes found.—A preliminary examination of soil samples from this orchard for nematodes again revealed a large nematode population, 80 per cent of which were *Xiphinema* sp. and closely resembled *X. americanum*. The pulling of several trees yielded no evidence of wooly apple aphid infestation. Eight representative trees in the orchard were then selected for a preliminary experiment in which 8 lb. of BHC suspended in water was sprinkled on 400 sq. ft. of soil surface around each of 4 trees on July 21, 1954. The remaining 4 trees were retained as untreated checks.

Response of apple trees related to nematode populations.—By fall, the foliage of the treated trees was much greener in color than that of the neighboring untreated trees. On November 4, 1954, soil samples were taken to a depth of 1 foot with a soil tube, 1 core being taken from under each of the 4 quadrants of the crown of each tree. These 4 cores were combined to make 1 composite sample for each tree. A 29-g portion of the composite sample was taken and processed by the Baermann funnel technique. After a 16-hr. period, the nematodes were drawn from the Baermann funnels into 50-ml beakers. The nematodes present in 3 low-power fields of a dissecting microscope were counted and the average of the 3 counts calculated. The results (Table 1) provide evidence that the nematode population about the apple tree roots in the treated areas was reduced in comparison to that around the untreated check trees (difference is significant at 5 per cent level).

TABLE 1.—*Relative numbers of nematodes found in soil samples from the roots of untreated and BHC-treated apple trees, 3 months after date of application*

Tree no.	Number of nematodes in low-power field of dissecting microscope Treatments	
	Untreated control	8 lb. BHC/400 sq. ft.
1	252	33
2	171	17
3	198	27
4	128	53

During the current season, no off-flavor could be detected in fresh fruits from the treated apple trees nor in apple sauce made from them. Fruits from treated peach trees were not tested for off-flavor in these experiments.

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ATTEMPT TO INCREASE BUNT EXPRESSION IN WHEAT BY UNUSUAL INOCULATION METHODS [1]

F. P. Zscheile[2]

SUMMARY

Attempts were made to obtain a large proportion of plants with symptoms of bunt, caused by *Tilletia caries* (DC.) Tul., in Baart, a susceptible variety of wheat, by unusual methods of inoculation. Mycelial cultures, germinated chlamydospores, and nongerminated chlamydospores were used. Seeds were germinated in contact with inoculum at low temperatures and were soaked or treated with nutrient media known to be beneficial to *T. caries* in cultures. Mycelial inoculum produced bunt under proper conditions of low temperature. Bunt expression was promoted by longer periods of contact of the seedlings with germinated chlamydospores at low temperatures. Results were promising in the greenhouse, where 89 per cent of inoculated plants developed bunt. Soaking of seeds in thiamine solution also promoted bunt expression. Injection of germinated seeds with inoculum was effective when germinated chlamydospores were used but not when mycelium was used. Injection of chlamydospores into the leaf whorl of young seedlings was most effective when the spores were suspended in a nutrient medium. Plants growing in the field were injected with inoculum at different stages of growth, from early seedling to when kernels were in the soft-dough stage. Two of 10 plants injected with chlamydospores above the apical node at the late tillering stage produced 1 bunted kernel each. When flowers on 5 plants were injected with chlamydospores, 3 flowers produced seeds having small sori of chlamydospores. No bunt was observed on plants of resistant varieties grown from seed or from seedlings inoculated by these methods.

Biochemical studies on the nutrient requirements of *Tilletia caries* (DC.) Tul. (10) showed that the progress of the fungus in the host plant might be dependent to a considerable extent upon certain available nutrients at various stages of growth prior to kernel maturation. In the present work, attempts were made to infect field-grown wheat plants in various stages of development with the pathogen suspended in different media and applied to different tissues in different manners. The substances native to the plants were supplemented with certain nutrients at the time of inoculation to determine whether or not such substances would stimulate growth of the fungus, promote vigorous infection, and cause better expression of bunt. These experiments extend the observations of Rabien (7), who studied the effects of both temperature and various inorganic substances on chlamydospore germination and infectivity. The possibility of breakdown of resistance in resistant wheat under favorable conditions of inoculations also was investigated.

Baart, a variety of wheat susceptible to *T. caries*, and either Baart 38 or Baart 46, two resistant varieties, were used in these studies.

STUDY OF MASS INOCULATION AT DIFFERENT TEMPERATURES.—Wheat seeds were germinated in cultures of *T. caries*, Race T-1 (3). Bunt balls were used to seed a minimal medium called MTO (10) in both agar and liquid forms. This medium contained inorganic salts and sucrose. Transfer cultures were made successively to produce cultures which consisted of growing mycelia and secondary sporidia and which were ultimately free of chlamydospores. For seedling inoculation, 30-ml portions of liquid media in flasks were seeded with third-transfer cultures. Prior to this seeding, the following were added to the medium in the indicated concentrations (ppm): glycine, 0.3; thiamine, 0.01; pyridoxine, 0.01; nicotinic acid, 0.05; and calcium pantothenate, 0.05. Incubation at 20° C produced fair growth in 24 days. Frequent shaking (by hand) of 2 flasks during this period resulted in retarded growth, a result not yet explained but readily demonstrable.

Inoculation of wheat seedlings.—Seeds of wheat were surface-sterilized with Chlorox (5 per cent sodium hypochlorite), washed in sterile water, and placed on plain agar plates. Germination occurred during 68 hours at 16° C, after which time the coleoptiles were 3 mm and the radicles 10 mm in length.

Flasks containing vigorous 24-day cultures of the fourth transfer of *T. caries* were shaken vigorously to break up the clumps of colonies. Then 108 seedlings per flask were immersed in the culture for 20 minutes, the liquid decanted, and the seedlings distributed uniformly on agar plates of the enriched MTO medium containing 1½ per cent agar. The agar from another plate culture was inverted over the plants in each case to maintain high humidity. Just prior to the plating, a second group of seedlings was inoculated with a heavy suspension in water of chlamydospores from sterilized bunt balls. A control group of Baart seedlings received no inoculation. All were incubated at 6°.

Eleven days after inoculation the seedlings were

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planted in moist soil in the field at Davis on December 10, 1948, 4–6 in. apart in rows 2 ft. apart. A similar series was planted in the greenhouse at the same time. Coleoptiles at that time were ½ in. and roots were up to 2 in. long. Microscopic examination of chlamydospores on the seed coats showed that the chlamydospores had not yet germinated.

From 200 field-grown Baart plants inoculated with chlamydospores, there was but 1 bunted plant, and among 200 Baart plants inoculated with the mycelial suspension, there were 11 bunted plants. In a parallel series in which 55 plants of Baart were inoculated with mycelia and grown to maturity in the greenhouse, 3 bunted plants were found. These bunted plants indicate that infection may occur from mycelial cultures under proper temperature conditions. Results of Sartoris (9) and Kienholz and Heald (5) were entirely negative on this point. No control plants showed evidence of bunt.

It thus appears that exposures to massive doses of mycelial inoculum, at the advanced seedling stage indicated, can produce successful infection and lead to bunt in susceptible wheat. This method does not surpass the usual method of dusting with chlamydospores before planting in the field, nor does it influence the expression of resistance of Baart 38. Apparently the low incubation temperature of 6° permitted the seedlings to develop sufficiently far enough ahead of the fungus so that successful invasion seldom occurred in the chlamydospore series.

Effect of germination temperature during inoculation.—In experiment 1, surface-sterilized seeds were kept moist at 29° C overnight. The swelling seeds, 110 per flask, were soaked 2 hours in a suspension of mycelium of *T. caries* which had been grown for 8 days on a liquid maximal medium, called MT1 (10). This medium contained sucrose and inorganic salts as found in MTO plus salts of manganese, zinc, and iodide plus asparagine, glutamic acid, and thiamine. The seeds were then transferred to agar plates of MT1 on which *T. caries* was growing vigorously from mycelial transfers. The mycelial suspensions used here were at least 38 transfers removed from their original chlamydospore source. Agar from similar plate cultures was inverted over them as indicated previously. Incubation at 16° followed for 3 days, after which a series was planted in the greenhouse and a parallel series in the field on December 17-19, 1949.

In experiment 2, a similar series of seedlings was treated as in experiment 1, except that germinated chlamydospores were used instead of a mycelial suspension. The spores were in contact with the seed during the initial overnight period at 16°, and no agar covered the seeds during the 3-day incubation period at 16°.

Experiment 3 was a duplicate of experiment 2 conducted with chlamydospores under different temperature conditions. The temperature varied between 4 and 16° for the first 48 hours of incubation, with a total time of 7 days at 16° or lower before planting in the greenhouse on January 11, 1950.

In experiment 4a, seeds were soaked in water at 4° until the coleoptile was barely emerging from the seed coat. Then they were transferred to a thick suspension of mycelial growth for 2 hours, after which they were placed between agar cultures of *T. caries* on MT1 medium as in experiment 1 and incubated at 4°. Experiment 4b was similar except that 1 per cent water agar was used instead of MT1 agar. In experiment 4c, seeds were treated with ungerminated chlamydospores and incubated moist at 4° for 3 weeks; then they were planted in the field February 13, 1950.

Plants for the field were kept at 4° after incubation for periods up to 2 days to retard growth until conditions in the field were appropriate for planting. The soil in the field was nitrogen-deficient. Of 317 plants of Baart inoculated by the usual method of dusting with chlamydospores, 66 per cent were bunted, with great variation among adjacent rows. This was equaled by experiment 2 in the field (Table 1).

Results in the greenhouse (Table 1) were particularly encouraging because bunt counts equal to those in the field had not heretofore been obtained under greenhouse conditions. The 89 per cent obtained in the greenhouse in experiment 3 was equivalent to the 83 per cent that was the highest obtained in that season for Baart inoculated in the field by the usual dusting procedure. Inoculation with chlamydospores and incubation at 4° (experiment 4c) gave only 30 per cent bunt incidence in the field, which is considerably lower than obtained when incubation occurred at 16° as in experiment 2. Results of these studies on effects of germination temperature during inoculation are in general agreement with those described by Rabien (7), who reported low temperatures (below 20° C)

TABLE 1.—*Percentage bunt in Baart wheat plants grown from seeds that were inoculated while germinating with different kinds of inocula and at different temperatures*

Exp.	Conditions of inoculation	No. of plants survived	Percentage of plants bunted
	Greenhouse-grown plants		
1	Incubation at 16° with mycelium for 3 days	92	0
2	Incubation at 16° with germinated chlamydospores for 3 days	106	53
3	Incubation at 4-16° with germinated chlamydospores for 7 days	97	89
	Field-grown plants		
1	Incubation at 16° with mycelium for 3 days	67	0
2	Incubation at 16° with germinated chlamydospores for 3 days	77	66
4a	Incubation at 4° with mycelium on MT1 agar for 2 weeks	36	3[a]
4b	Same as 1, except for 1% water agar instead of MT1 agar	47	0
4c	Incubation at 4° with nongerminated chlamydospores for 3 weeks	31	30

[a] Six of 12 heads were bunted on this plant.

favorable to high incidence of bunt only when seeds that were germinated with chlamydospores were held at these temperatures for a long period of time.

INFECTION OF SEEDLINGS BY MECHANICAL INOCULATION.—A very high percentage of infection by cultures of *T. caries* was not obtained in the preceding studies when seedlings were inoculated at 4–16° C and later grown in the field or greenhouse. A further experiment was designed to determine whether or not actual insertion of the inoculum into the tissues of the young seedlings will result in higher percentages of infection.

Seeds were prepared as above and germinated on wet filter paper at 20° for 4 days. Suspensions of mycelium were dispersed by repeated forceful expulsion of a 10-day-old culture through a 19-gauge hypodermic needle. Chlamydospores, previously germinated for 4 days at 20° on water-agar slants, were washed from the agar in sterile water. Suspensions of both types of inoculum were also made in medium MT1 (10). Several methods of inoculation were used (Table 2). Usually 10 plants were treated by each method. These were then planted in the greenhouse or field for growth to maturity.

Baart 38 survived these inoculations about as well as Baart. It is apparent that the thread treatment is too drastic to be practical, especially with chlamydospores (Table 2). Possibly chlamydospores caused such severe infection that the plants did not survive the seedling stage. The same treatment with mycelium did not cause such a high mortality rate. Most plants produced heads, but not all survived because of certain accidental causes. Of the small number of heads involved, however, no bunted plants resulted from any injection with mycelium. Germinated chlamydospores were very effective in the seed and nongerminated chlamydospores were effective in the growing-point region within the plumule. This demonstrates that infection can occur at still later stages of seedling growth than those studied in the first experiments, and that it can occur in plant parts above ground as well as in those under ground. These observations are in agreement with results of Bodine and Durrell (2) with *T. levis*. Whether MT1 was superior to water as a dispersing medium cannot be decided because of the small numbers of survivals.

TABLE 2.—*Results of mechanical inoculation of Baart wheat seedlings with the bunt fungus*

Method of inoculation	No. of plants survived	No. of heads obtained	No. of plants bunted
Inoculum in water			
Injection of mycelium[a]	5	1	0
Injection of germinated chlamydospores[a]	10	5	5
Thread with mycelium[b]	6	5	0
Thread with mycelium[b]	0		
Mycelium in leaf whorl[c]	10	4	0
Nongerminated chlamydospores in leaf whorl[c]	10	4	2
Inoculum in MT1[d]			
Injection of mycelium[a]	10	1	0
Injection of germinated chlamydospores[a]	10	3	1
Thread with mycelium[b]	6	2	0
Thread with chlamydospores[b]	0		
Mycelium in leaf whorl[c]	10	3	0
Nongerminated chlamydospores in leaf whorl[c]	11	10	8

[a] Injections of these suspensions were made with a 26-gauge hypodermic needle into the seed as close to the growing point as possible; planted in greenhouse.

[b] Darning thread, soaked in the suspension, was drawn laterally through the growing coleoptile with a needle, and a short piece of thread was left in the seed; planted in greenhouse.

[c] Fifteen plants, planted December 2, 1949, were grown 5 weeks in flats outdoors. They were inoculated by injection of inoculum into the interior of the leaf whorl from above by insertion of a 22-gauge needle as far as it would easily penetrate. Three weeks later these plants were transferred to the field.

[d] A maximal medium favorable for growth of *T. caries* (10).

TREATMENT OF SEEDS TO FACILITATE INFECTION BY THE BUNT FUNGUS.—It was considered possible to enhance the infection rate and hence the final bunt count by addition of nutrients to the seedling for the nourishment of the invading fungus. To this end the treatments described below were tried.

Treatment 1.—One hundred ten surface-sterilized seeds were soaked in liquid MT1 medium at 4° C. After 40 hours, a 10-ml suspension of germinated chlamydospores, prepared as in the previous experiment, was added. Eight hours later the temperature was raised to 30° for 24 hours after which the seedlings were planted in the field on December 21, 1949.

Treatment 2.—The conditions were the same as in treatment 1 except that seeds were soaked in a solution containing 100 ppm of thiamine instead of medium MT1.

Treatment 3.—Seeds were treated with germinated chlamydospores overnight at 20°, then placed on agar plates at 16° for 6 days to germinate. Coleoptiles were ½ in. long. Then they were injected with medium MT1 by means of a 26-gauge hypodermic needle inserted into the middle of the seed. Enough medium was forced into the seed to cause bursting of the outer seed coat. They were planted in the field on December 20, 1949.

Treatment 4.—Conditions were the same as in treatment 3 except that a thiamine solution containing 100 ppm was used instead of MT1 which contains only 1 ppm thiamine.

Treatment 5.—One hundred ten seeds were soaked in liquid MT1 medium at 4° for 72 hours, then inoculated with a suspension of nongerminated chlamydospores and placed on water agar plates at 4–7° for 3 weeks, after which they were planted in the field on February 13, 1950.

Treatment 6.—Conditions were the same as in treatment 5 except for the use of 100-ppm thiamine solution instead of medium MT1.

TABLE 3.—*Percentage of bunted plants of the Baart variety grown from inoculated seed soaked in or injected with various nutrients*

Treatment	No. of plants survived	Percentage of bunted plants
1. Seeds soaked in MT1[a] with germinated chlamydospores	82	18
2. Seeds soaked in thiamine solution with germinated chlamydospores	90	14
3. Seeds injected with MT1 after germination with chlamydospores	70	6
4. Seeds injected with thiamine solution after germination with chlamydospores	82	1
5. Seeds soaked in MT1 with nongerminated chlamydospores	36	19
6. Seeds soaked in thiamine solution with nongerminated chlamydospores	35	55[b]

[a] A maximal medium favorable to growth of *T. caries* (10).
[b] All heads of bunted plants were completely smutted.

Treatment 1 to 4 provided chlamydospores with a head start before they contacted the seedlings, and supplemented the nutrients normally available to the fungus.

Survival rates were high, and fair bunt counts were obtained with Baart (Table 3). Treatments 1 and 2 produced higher bunt counts than 3 and 4, indicating that soaking the seeds in nutrients is more favorable to infection than is injection of nutrients. Results of treatments 5 and 6 suggest that possibly thiamine might be a factor in the nutrition of *T. caries* on or in young seedlings. The numbers of surviving plants were small. The low temperature also may have contributed to the higher figures for treatments 5 and 6, although the experiments on mass inoculation at different temperatures suggest that such is not the case. The highest bunt count obtained, 55 per cent, compares favorably with that obtained by conventional dusting methods, but none of these special treatments appear to offer any improvement.

ATTEMPTS TO INFECT OLDER PLANTS WITH T. CARIES.—A study was made in early 1950 of the stages of development at which field-grown plants of Baart and Baart 46 might be successfully inoculated with *T. caries* by injection methods. Suspensions of germinating chlamydospores and of mycelia of vigorous cultures of *T. caries* in MT1 were injected by means of a hypodermic needle into 2 series of plants growing at a uniform rate on fertile soil. Plants or tillers were labeled and were examined carefully at maturity for evidence of bunt, both at the point of inoculation and within the individual kernels in the head. The experimental program is outlined in Table 4. In younger plants, heights were measured to leaf extremities.

No bunt developed in any of these plants except in a few cases of Baart inoculated with chlamydospores. Two plants inoculated above ground-level above the apical node on March 30 had 1 bunted kernel each. Three kernels inoculated individually April 25 had 1 or more sori of chlamydospores just under the epidermis; 1 of these kernels was half displaced by spores, far in excess of possible dried-up inoculum; 1 had a sorus 1 mm^2 in size; and 1 had 4 smaller sori. One kernel inoculated on April 27 had a bunt sorus that appeared to have produced many chlamydospores, some of which were stained by cotton blue (8), an indication of their immaturity. Kernels inoculated later than April 27 did not develop bunt.

In similar tests, Bodine and Durrell (2) observed

TABLE 4.—*Incidence of bunt following inoculation by various methods during different stages of wheat development*

Date inoculated	Stage of development of wheat	Location of puncture	Incidence of bunt following inoculation with[a]	
			Chlamydospores	Mycelium
3/14	Early seedling	At ground level	0/10	0/10
3/21	Early tillering	½–1 in. above ground	0/10	0/10
3/30	Height 16-18 in.; late tillering	Just above ground and probably below growing point	0/9	0/10
		3 in. above ground and 1 in. above apical node, aimed at young head and growing point	2/10	0/10
4/6	Height 24 in.	0–1 in. above ground, in stem	0/10	0/10
		6–12 in. above ground, 2–3 in. above apical node aimed at young head	0/10	0/10
4/14	Head 1 in. long	Aimed at head	0/10	0/10
4/25	Heads still in boot	Head area in boot flooded from base	0/5	0/5
4/25	Heads out of boot	Injection of each flower at its base, along 1 side of head only	3/5	0/5
4/27	Heads out of boot, but not in bloom	Base of each flower punctured and flooded with inoculum	1/8	0/10
5/4	In bloom	Base of each flower punctured and flooded with inoculum	0/8	0/9
5/9	In bloom	Base of each flower punctured and flooded with inoculum	0/10	0/10
5/16	In milk stage	Base of each kernel punctured and flooded with inoculum	0/9	0/9
5/23	In soft dough stage	Base of each kernel punctured and flooded with inoculum	0/8	0/9

[a] Numerator is number of bunted kernels; denominator is number of inoculated plants.

no infection following inoculation of heads by injection of flowers or nearly mature heads with cultures or spore suspensions of *T. levis*, although at earlier stages of development bunted heads resulted from injection methods. Milan (6) successfully inoculated young culms, wounded at their bases, with chlamydospores, but Ajroldi's attempts (1) were successful only at early stages of development.

Most punctured kernels were shriveled when mature. All other kernels developed well. One hundred kernels later were stained with cotton blue and examined for possible chlamydospores developed within the kernel's interior (4). Both Baart and Baart 46 that were inoculated after April 25 were examined. No such chlamydospores were found. All punctured kernels of Baart 46 inoculated with mycelium were carefully examined under the binocular microscope, but no evidence of chlamydospore formation was found.

These unusual methods do not appear promising for inoculation procedures, at least under field conditions in Davis. None of the parallel experiments conducted with resistant wheat varieties, Baart 38 and Baart 46, produced any bunt symptoms.

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A STUDY OF MYCELIAL MATS OF OAK WILT[1]

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SUMMARY

A study of mat formation by *Endoconidiophora fagacearum* Bretz, the fungus causing oak wilt, in Minnesota during 1953 and 1954 showed that on trees wilting in July, mats formed in August, September, or October. In most trees, mat formation was completed before the following spring. Few mats were produced and the period of mat formation was of short duration on trees that began to produce mats during hot weather. Mats were formed more abundantly and the duration was longer (8 weeks average) when they did not start to form until late September or October, when weekly mean temperatures ranged from 55–65°F. Trees that wilted in August did not produce many mats until the following spring. Trees wilting in September failed to produce mats or produced them for a short period of time late in the following spring. Low precipitation did not prevent mat production on standing wilted oaks. Numerous mats were formed around, and adjacent to, small wounds made through the bark soon after the trees wilted. Temperatures of 68–81°F (20–27°C) appeared to be optimum both for formation of large numbers of perithecia and for their rapid maturation *in vitro*.

Mycelial mats of *Endoconidiophora fagacearum* Bretz, the cause of oak wilt, were first discovered in 1951 (5). Leach et al (13) showed that these mats were a mechanism for liberating spores from beneath the bark. Insects may carry inoculum from these mats to wounds in healthy trees (7, 10, 12, 14, 15) and thus spread the disease.

The purpose of the present study was to determine the seasonal prevalence of mycelial mats and the effect of environmental conditions on mat formation. This information should contribute to our knowledge of the epidemiology of oak wilt and might possibly be useful in some aspects of control of the disease.

There is little information on the factors affecting the production of mats. Trees bearing fresh mats in Illinois had been dead for approximately 3 months to 1 year (6). In Pennsylvania, the length of time required for mat formation after symptoms of wilt developed varied, but it was especially dependent on the month during which the above ground parts died (8). If death occurred from April to June, mats might form from August through November of the same year. If death occurred in July or later, the mats developed in late winter or early spring of the following year. Fergus (8) also reported that the sapwood moisture content of 3 trees with fresh viable mats was lower than that of 3 other trees that were wilted but still had green cambium and no mats. The moisture content of the sapwood beneath mats in October and November in Minnesota was approximately equal to that of the outer heartwood, while in healthy trees the sapwood moisture content was 5.4 per cent lower, wet-weight basis, than that of the outer heartwood (9).

Mycelial mats have been reported as occurring during various times of the year. In Illinois, mats were found during the autumn and winter of 1951 and spring of 1952 (6). Later, Curl (4) concluded that the greatest concentration of potential inoculum could be expected in March, April, and May. In Pennsylvania, mats were found in most of the months from March to November (8).

In nature, perithecia of *E. fagacearum* are produced on mycelial mats (2, 16, 18). As yet, the relative importance of ascospores and of conidia in the overland spread of the disease is not known. Transmission of the pathogen occurred when the vectors were in contact with perithecium-bearing mats or cultures (7, 10, 12, 15). It has been shown recently, however, that conidia alone may be insect transmitted (14).

In the present work, the effect of temperature on the development of perithecia was studied *in vitro* so that it might be related to perithecial formation on mats. Stambaugh et al (17) reported that the optimum temperature range for maximum perithecial formation and ascospore extrusion was 18–24°C on maltose-casamino acids agar.

MATERIALS AND METHODS.—Oak wilt can readily be recognized by foliar symptoms from mid-June to mid-September in Minnesota. In 1953, wilting trees were tagged at intervals from July to mid-September. On subsequent visits, which continued until December, notes were taken on the condition of these trees and on mats which formed. The area studied was located on level land about 25 miles northwest of St. Paul, Minnesota, in the sand outwash area. The stand consisted of second growth northern pin oak (*Quercus ellipsoidalis* E. J. Hill) with a few scattered bur oak (*Q. macrocarpa* Michx.). Oak wilt had been present in this woodlot for several years and was present in most of the nearby oak stands. The weather data were taken from records of the U. S. Department of Commerce Weather Bureau for the Minneapolis-St. Paul airport, the closest weather station, which was about 24 miles to the south.

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Data were recorded on 39 northern pin oaks from 3–9 in. D.B.H. (diameter at breast height). Data obtained from trees tagged during each individual month have been studied as a group. In most cases foliar wilt occurred during the month the trees were tagged and continued into the following month.

MAT FORMATION.—Mats are not formed immediately when a tree wilts. The cambium and phloem remain green and healthy-appearing for a time. Later they become light brown, and the outer surface of the sapwood appears watersoaked. Often the odor characteristic of the fungus mats is pronounced at this time. On the trees observed, the development of these symptoms progressed from the top downwards. Mats are formed soon after the cambium turns brown. After the mats have formed, the cambium, phloem, and sapwood become dark brown to black and foul-smelling, after which no more mats develop.

The time from complete wilt to the formation of the first mats on trees wilting in July varied from less than 1 to as long as 9 weeks, with an average of $4\frac{1}{3}$ weeks. This interval from complete wilt to initial mat formation was not correlated with the date of complete wilt or with the diameter of the tree.

Mats were found from ground line up into the crown where they appeared on the larger branches. At any one time, however, most of the mats were found in a zone which extended approximately 10–15 ft. along the bole. There, mats ranged from young to mature. Young mats had abundant endoconidia, and often perithecia had developed on the older, mature mats. Formation of mats began in the upper part of the bole and progressed downward. Thus, mats in various stages of development were found on the same bole.

The descriptions of mycelial mats in the literature do not portray them as part of a definite step-wise pattern. This pattern was found to be characteristic of the red oaks studied, with variation in the time required for its completion and in the number of mats produced. Mats are formed for a relatively short time when conditions are favorable for their production, and constitute the final phases of the disease in the aboveground parts of the host tree.

Seasonal pattern of mat formation.—Mat formation on 16 of 18 trees wilting in July was completed the same fall (Fig. 1,A). Additional mats were formed in May, 1954, on 2 trees which bore mats in October and November, 1953. In contrast, the 12 trees that wilted in August, 1953, formed some mats in the fall of 1953 but most were formed in May, 1954, and a few in June. No mats were formed between December 1, 1953, and April 8, 1954, as might be expected since the temperature is seldom above freezing during this period.

The effect of seasonal temperatures also is shown. The peak of mat formation in the fall occurred when the weekly mean temperatures were approximately 55–65°F, and in the spring when the temperatures were 41–53° (Fig. 1, A). Five weeks intervened between the first examination of these trees in April and the second examination when mats were found so that the exact time of their formation is not known. It is possible that these mats were formed in mid-April when the weekly mean was at 53°F, and that the mats were in good condition when observed in May because of cool weather.

Duration of mat production on individual trees.—The data on duration of mat production on the trees wilting in July, 1953, was analyzed statistically because most of the mat formation occurred during the same season as the wilt. Since these trees were not all in the same stage of wilt at the same time, they were classified as to the time at which they were completely wilted. Trees were considered completely wilted when all the foliage had turned brown.

The later the occurrence of complete wilt, the longer was the duration of mat production (Fig. 1, B). Two trees produced additional mats the following spring, hence the line probably should be somewhat higher. The correlation coefficient, calculated on the basis of the duration of mat formation in 1953, was $r = 0.65$ and was significant at the 1 per cent level.

No correlation was found between tree size and the duration of mat production, probably because the trees were all in the small-size class and there was little variation within the group.

Since the time from complete wilt to mat formation was extremely variable, the duration of mat production was plotted against the date when the first mats were observed (Fig. 1, C). The last average is low for it includes 2 trees that produced additional mats the following spring. When the mat-producing stage is not reached until cool fall temperatures prevail, the duration of mat production is greater than on trees that begin to produce mats in the summer. The cold winter temperatures, of course, reduce the mat-producing period; however, in some cases, more mats are produced the following spring when the temperature again becomes favorable.

Drouth did not prevent the formation of mats on standing trees as evidenced by the presence of mats during the dry falls of 1952 and 1953. Mat formation during the drouth in the fall of 1952 has been reported (3). In 1953, the August precipitation was approximately normal, 2.75 in.; however, the precipitation was 0.70 in. from September 1 to November 19, when most of the mats were produced on trees that wilted in July. This was a departure of 3.80 in. below normal for September and October.

Eight trees were studied on which wilt symptoms developed in September, 1953. The leaves on these trees were becoming yellow and red. This color change resembled fall coloration except that it occurred about 3 weeks early. The cambium on all these remained green until December 1, 1953, and on 6 until May 11, 1954. Sprouts appeared on the trees of the latter group in June, 1954, and persisted until July. When the sprouts wilted, the cambium quickly became brown and was soon past the stage suitable for mat formation. One mat was found on each of 2 of these trees in July and early August, 1954. Two mats were found

on May 24, 1954, on 1 of the trees which had brown cambium on May 11. Apparently the temperature was too warm, above 70°F weekly mean, for effective mat formation by the pathogen when these trees reached the mat-forming stage in June and July.

Mat formation near openings in bark.—Although mat formation on the trees observed in this study was not associated with wounds, some observations were recorded on the relation of mat formation to artificial wounds. Fergus (8) observed that mats formed more abundantly near the cut ends of logs and billets (bark left intact) than in central portions of the logs. He suggested that this might be due to a reduction in moisture content near ends exposed to the air.

Twenty-three mycelial mats were found on 8 trees near cuts made with an ax (Fig. 2). These cuts had been made between the time the cambium began to turn brown and the time mats were formed. No mats were formed near cuts made before this stage. Mats also were formed at the edge of openings around other mats where the bark overlying the mat had been removed, or where the mats and underlying wood were

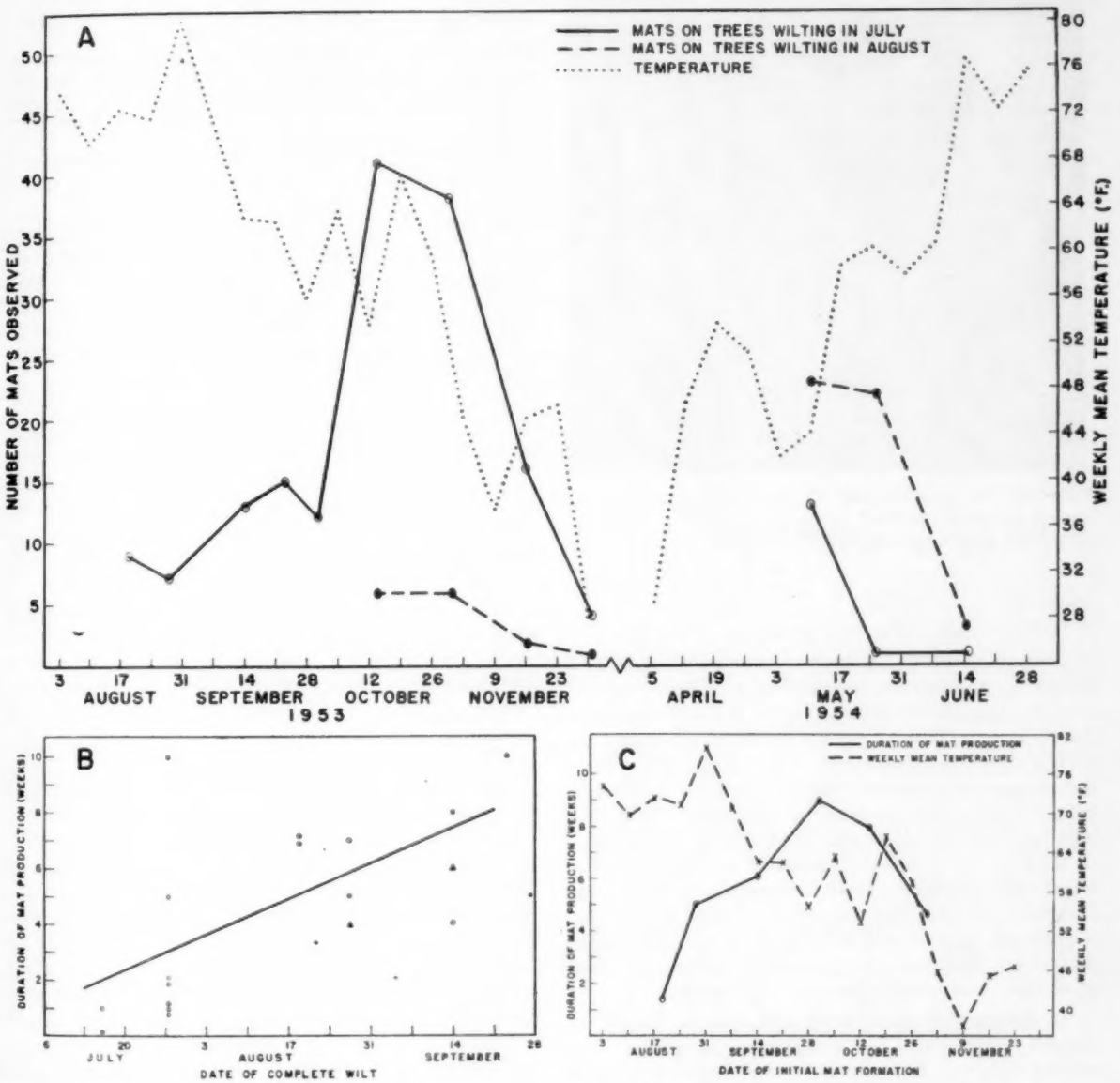

FIG. 1. A) The seasonal appearance of mycelial mats of *Endoconidiophora fagacearum* Bretz on northern pin oaks wilting in July and August, 1953, as related to weekly mean temperature of the week preceding mat formation. B) Regression line of the date of complete wilt and the duration of mat production on 18 trees wilting in July, 1953. Two solid triangles indicate 2 trees on which additional mats were produced in 1954. C) The relation of the duration of mat production to the date of initial mat formation and to weekly mean temperature for trees wilting in July, 1953.

FIG. 2. The mycelial mat and pressure cushion formed around an ax cut (center) made 13 days earlier. Bark removed for photograph on October 13, 1953.

removed.

Effect of temperature on formation of perithecia.—In July, 1953, studies were made on the effect of temperature on formation of perithecia. Three isolates, 2 of A compatibility type and 1 of B type, were used. Acorn agar (11) was used with duplicate plates for each temperature treatment. The 7 temperature treatments were as shown in column 1 of Table 1. The receptor culture was implanted at the center of the plate and incubated for 2 weeks. At this time endoconidia of the opposite type were harvested in sterile distilled water from approximately 2-week-old cultures and sprayed onto the receptor culture with a sterilized hypodermic syringe and needle. Five days after spermatization, the plates were examined and the numbers of mature exuding perithecia were counted.

An analysis of variance of the results (Table 1) showed significance for temperature treatments but not for isolates. Few perithecia matured at room temperatures, which were similar to those occurring in the field during July, 1953. It is of significance, however, that the colonies incubated at room temperatures for 2 weeks formed abundant perithecia when held at 25°C after spermatization.

TABLE 1.—*The average numbers of perithecia of Endoconidiophora fagacearum formed on duplicate plates of acorn agar under various temperature treatments, 5 days after spermatization on acorn agar*

Temperature treatment[a]	Number of perithecia produced by 3 isolates			
	1	2	3	Mean[b]
15°—15°	0	31.5	4	11.8
20°—20°	0	218	93.5	103.8
25°—25°	203	121	145	156.3*
Room—Room	7	0.5	0	2.5
Room—15°	3	79	91.5	57.8
Room—20°	176.5	113.5	130	140.0*
Room—25°	128	231	115.5	158.2*

[a] Temperature before spermatization and temperature after spermatization, respectively. Numbers indicate °C. The room temperature before spermatization averaged 26° with a range of 22.5 to 31°; after spermatization it averaged 27.6° with a range of 25.6° to 30.5°.

[b] The symbol * indicates significance at 5 per cent level for the means.

Fewer perithecia were produced when similar tests were made on potato-dextrose agar and on maltose casamino acids agar (1). Temperature relations were similar to those on acorn agar. The poor results with the maltose casamino acids agar were probably due to a faulty formulation of the medium, and to the fact that only 1 implant was made per plate.

In an experiment on the time required for maturation of perithecia at various temperatures, 2 isolates (1 each of A and B types) were implanted on plates of chestnut agar and incubated at 25°C for 19 days. Following spermatization with compatible endoconidia, duplicate plates of each isolate were placed in constant-temperature chambers at 8°, 12.5°, 20°, 25°, 27°, and 30°C and inspected twice daily for perithecia.

Immature, nonexuding perithecia were seen when they had formed a globose base and at least a short neck. The perithecia were considered as mature when the ascospores were exuded, although mature ascospores no doubt were present within the perithecium before then.

The time from spermatization until exuding perithecia were formed ranged from 58 hours at 25° to 339 hours at 8° for 1 isolate, and from 58 hours at 27° to 407 hours at 8° for the other isolate. Exuding perithecia were formed within 3 days by both isolates at 20° to 27°. No perithecia formed in 219 hours at 30°. The time interval from the first observation of immature perithecia to observation of exuding perithecia ranged from 10 hours at 25–27° up to 108 hours at 8°.

After 400 hours perithecia were abundant at temperatures of 20° to 25° and almost as numerous at 12.5° and 27°, whereas few formed at 8°.

Considering the rapidity of perithecial maturation and the numbers of perithecia formed, it is concluded that temperatures of 20° to 27°C are most favorable for the formation of perithecia *in vitro*, which agrees well with previously reported work.

The practical result of the retarding effect of high

temperatures on perithecial development was demonstrated with mats formed in nature during hot weather in late August, 1953, when the weekly mean temperature was 26.7°C. A number of immature perithecia were observed on these mats when they were brought to the laboratory. The mats evidently had been spermatized, as the perithecial bases had formed and the necks were beginning to elongate. The mats were wrapped in wax paper; some were placed at 25°C and the remainder were left at room temperature. An abundance of exuding perithecia were produced after 2½ days at 25°. Those at higher room temperatures, maximum of about 29–34°C, had not begun to exude.

Mats with an abundance of perithecia have been found in late May to early June and in October to November in Minnesota. In August and September perithecia were found on some mats but were not as abundant as late in October.

Discussion.—In Minnesota in 1953-54, *E. fagacearum* produced mats at 2 rather distinct periods. One of these was from August through November, primarily on trees wilting in July and to some extent on trees wilting in August. During this period, the conditions within the recently wilted trees were usually favorable for mat formation. If the temperature was favorable, as it was from mid-September through October, mats were produced abundantly. It is quite possible that in some years few or no mats would be formed after the early weeks in October because of an early winter. Trees wilting in June probably bear mats during the early part of the fall period, but no data are yet available. The other period was in May and early June, during which time mats were produced on trees that had wilted the previous August and on a few that had wilted in July and September. Conditions within the trees after overwintering are not as favorable for mat production, however, as in the fall. Two internal conditions which appear to be important are the moisture content of the host sapwood and the presence of other antagonistic microorganisms. Few mats formed from late June through July, and those that did appeared to deteriorate quickly.

The formation of mycelial mats apparently is greatly influenced by high temperature that may act directly on the fungus by reducing its growth rate within the host, or indirectly by increasing the rate of desiccation so that the sapwood becomes too dry for development of mats. High temperatures do not appear to restrict the activity of other microorganisms and insects which are found on mats and which possibly are responsible for their deterioration.

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MUTATIONS FOR STEM RUST RESISTANCE INDUCED IN OATS BY X-RAY TREATMENT[1]

K. J. Frey and J. A. Browning[2]

SUMMARY

A number of mutations that conditioned resistance to races 7, 7A, and 8 of the oat stem rust fungus were found in irradiated Huron oats, but none was found that gave resistance to race 6. Genetic data showed that at least 2 of the mutant lines possessed the Richland gene for stem rust resistance. The pathologic data suggested that other mutant strains that were resistant to race 8 possessed the White Tartar gene. Only 1 strain of the 61 tested appeared to contain a mutation for stem rust resistance that was different from known genes

One of the most interesting mutation types obtained by treating the cereal crops with ionizing radiation has been that for disease resistance. The first such mutation was reported by Freisleben and Lein (3) who selected barley strains resistant to mildew from an irradiated susceptible variety. More recently, mutations that induce stem rust resistance have been isolated from irradiated oats by Frey (2, 4) and Konzak (6). MacKey (7) also reported the finding of similar mutations from wheat that had been treated with thermal neutrons. In each case the segregation within the irradiation derived lines seemed to follow that expected from a single-factor pair.

This paper is a more comprehensive report of previous articles by Frey (4) and Browning and Frey (2) in which brief descriptions were given of the mutations obtained from an irradiation experiment with oats. The material is presented in 2 parts: 1) pathological data for a few selected mutant strains of oats to illustrate the types of mutations for stem rust resistance obtained, and 2) the segregation for reaction to specific races of stem rust in crosses of the mutant oat strains with certain commercial varieties. Only the mutations for disease resistance will be discussed in this paper, since a companion paper describing the agronomic mutations is being published elsewhere (5).

MATERIALS AND METHODS.—The Huron variety of oats, which is tall, weak strawed, midseason in maturity, and susceptible to all known races of the oat stem rust fungus, was used for the irradiation experiment reported herein. Four hundred primary seeds, containing about 9½ per cent moisture, and irradiated with 25,000 r-units of X-rays, were planted in the field at East Lansing, Michigan, in 1950. Approximately 45 per cent of the irradiated seeds produced mature plants. Each X_1 plant was harvested and threshed separately, and in 1951 one row containing 25 spaced plants was planted from each X_2 progeny. The X_3 progenies from selected X_2 plants were sown in plant rows in 1952 and the 61 mutant strains that appeared to breed true were grown in yield tests at Ames, Iowa, in 1953 and 1954. In each year a severe epiphytotic of oat stem rust, predominantly race 7, developed, resulting in a differentiation of the mutant strains into 2 classes, those resistant and those susceptible to stem rust.

Seedlings from each of the 61 mutant strains were tested for reaction to races 6, 7, 7A, and 8 of the oat stem rust fungus (*Puccinia graminis* Pers. f. sp. *avenae* Eriks. & E. Henn.) Seedlings in the first leaf stage were sprayed with a 1 per cent solution of Tween-20[3] in distilled water, inoculated by dusting with spores diluted with talc, incubated for 15 hours in a moist chamber at about 70°F, and moved to a greenhouse maintained at 70°. Readings were made on the twelfth day following inoculation, using the infection types described by Bailey (1). Plants with a 0-, 1-, or 2-type reaction were considered to be resistant and those with a 3- or 4-type reaction as susceptible. The classification of a mutant strain as resistant, segregating, or susceptible to a given race of the fungus was usually based upon readings from 70 or more seedlings.

Several crosses were used to study the nature of inheritance of the mutations for stem rust resistance. The stem rust reactions of the F_2 seedlings from these crosses were obtained as previously outlined.

EXPERIMENTAL RESULTS.—*Reactions of mutant lines.*—The reactions of 4 families[4] of mutant oat strains to 4 races of the stem rust pathogen are given in Table 1. None of the selections was resistant to race 6. The reactions of the mutant strains to races 7 and 7A were parallel, with but 1 exception, strain A746-2-7. This strain appeared to be segregating for resistance to race 7 but was susceptible to race 7A. If this is true, it indicates the existence of a heretofore unknown gene conditioning resistance to the oat stem rust fungus.

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[2] Associate Professor of Farm Crops and Assistant Professor of Botany and Plant Pathology, respectively.

[3] An emulsifier (sorbitan monolaurate polyoxyethylene derivative) manufactured by the Atlas Powder Co., Wilmington, Del.

[4] A family refers to the group of mutant strains derived from 1 irradiated seed.

TABLE 1.—*Reactions of selected mutant oat strains to the oat stem rust fungus*

Selection	Stem rust race[a] 6	7	7A	8	Field reading[b]
A 746-2-1	S	Seg	Seg	Seg	5
-3	S	S	S	R	10
-5	S	R	R	S	1
-7	S	Seg	S	Seg	5
-8	S	S	S	R	5
A 746-7-2	S	S	S	Seg	10
-4	S	S	S	Seg	30
-5	S	S	S	Seg	30
-6	S	R	R	S	1
A 746-23-2	S	R	R	S	1
-3	S	Seg	Seg	Seg	2
A 746-45-7	S	R	R	S	3
-8	S	R	R	S	1
-9	S	R	R	S	1
Huron	S	S	S	S	20
Clintland	S	S	S	R	10

[a] "S" indicates a susceptible reaction, "R" a resistant reaction, and "Seg" that the selection was segregating for resistance to the indicated race of the fungus.

[b] Coefficient of infection, calculated as the product of the infection type (read on a scale of 0 to 1, in tenths, with 1 indicating complete susceptibility) and the percentage of the plant's stem surface covered with uredia. The field readings were made in 1954 in replicated yield plots.

The pathologic reactions of the other 4 strains in this family can be explained very readily on the basis of known genes for stem rust reaction. Strain A746-2-1 could possess a combination of the Richland and White Tartar genes, A746-2-3 and A746-2-8 could contain the White Tartar gene in the homozygous condition, and A746-2-5 could have the Richland gene in the homozygous condition. In family A746-7, 3 of the lines were segregating for reaction to race 8, and the other was resistant to races 7 and 7A. A similar situation was found in family A746-23, where 1 strain was resistant to races 7 and 7A, and the other was segregating for reaction to races 7, 7A and 8. All 3 strains in family A746-45 were resistant to races 7 and 7A. It would appear from the pathological data that at least 2, and possibly 3, different genes conditioning reaction to stem rust were mutated in the irradiated oat seeds.

A summary of the reactions of the 61 oat strains to 4 races of the oat stem rust fungus is given in Table 2. Over 75 per cent of the lines were either resistant or segregating for reaction to races 7 and 7A, whereas only 6 lines were resistant to race 8. There was either an unintentional selection for lines that were resistant to race 7, or the mutation conditioning resistance to this race occurred at a faster rate than the one producing resistance to race 8.

Segregation for stem rust reaction.—In order to study the inheritance of reaction to oat stem rust in the mutant strains, a number of crosses were made between selected strains resistant to stem rust and the commercial varieties Huron, Richland, and Eaton. The pathologic reactions of the parents and F_2 seedlings from these crosses to races 7, 7A, and 8 are given in Tables 3, 4, and 5, respectively. In the 2 crosses involving a resistant mutant and Huron, the segregation of the F_2 plants gave a satisfactory fit to a ratio of 3 resistant to 1 susceptible, indicating the presence of 1 factor pair conditioning resistance to race 7.

TABLE 2.—*Numbers of mutant oat strains resistant, susceptible, and segregating for reaction to 4 races of the oat stem rust fungus*

Reaction	Stem rust race 6	7	7A	8
Resistant	0	45	44	6
Segregating	0	3	3	8
Susceptible	61	13	14	47
Total	61	61	61	61

Richland and Eaton varieties of oats have been shown to contain the Richland gene, which produces resistance to races 7 and 7A but not to race 8. In the crosses of the resistant mutants by either Richland or Eaton, it would be expected that if the Richland gene were present in the mutant strains, all F_2 plants would be resistant to races 7 and 7A. No F_2 plants susceptible to races 7 or 7A were found in the crosses involving Eaton and Richland (Table 3, 4), an indication that the mutated gene for stem rust resistance in lines A746-35-6 and A746-47-6 was the same as the Richland gene.

In the crosses of the resistant mutants and Huron (Table 4), the segregation of F_2 plants for reaction to race 7A gave a satisfactory fit to a ratio of 3 resistant to 1 susceptible. Apparently, 1 gene pair was segregating for reaction to race 7A in each of these crosses.

In the cross of Huron × A746-40-6 (Table 5), the F_2 plants segregated for reaction to race 8 according to the ratio of 3 resistant to 1 susceptible, an indication that 1 factor pair was segregating for resistance to race 8 in the mutant strain of oats.

DISCUSSION.—There are 2 extremely interesting aspects resulting from the study reported herein. First, at least 2 mutations that condition resistance to oat stem rust were found in the irradiated material. Two

TABLE 3.—*Reactions of F_2 plants in 4 oat crosses and of parental strains to race 7 of the oat stem rust fungus*

Cross or parent	Reaction Resistant	Susceptible	χ^2
Huron	—	X	
Eaton	X	—	
Richland	X	—	
A 746-33-6	X	—	
A 746-35-6	X	—	
A 746-47-6	X	—	
A 746-55-1	X	—	
Huron × A 746-55-1	24	11	0.48[a]
Huron × A 746-33-6	41	10	1.04[a]
Richland × A 746-35-6	138	0	
Eaton × A 746-47-6	122	0	

[a] χ^2 value required for significance at the 5 per cent level = 3.84.

TABLE 4.—*Reactions of F_2 plants in 4 oat crosses and of the parental strains to race 7A of the oat stem rust fungus*

Cross or parent	Reaction		χ^2
	Resistant	Susceptible	
Huron		X	
Eaton	X		
Richland	X		
A 746-33-6	X		
A 746-35-6	X		
A 746-47-6	X		
A 746-55-1	X		
Huron × A 746-55-1	19	5	0.21[a]
Huron × A 746-33-6	35	16	1.04[a]
Richland × A 746-35-6	194	0	
Eaton × A 746-47-6	220	0	

[a] χ^2 value required for significance at the 5 per cent level = 3.84.

TABLE 5.—*Reactions of Huron, A 746-40-6, and F_2 plants in the cross Huron × A 746-40-6 to race 8 of the oat stem rust fungus*

Cross or parent	Reaction		χ^2
	Resistant	Susceptible	
Huron		X	
A 746-40-6	X		
Huron × A 746-40-6	38	11	0.12[a]

[a] χ^2 value required for significance at the 5 per cent level = 3.84.

of the mutant strains were shown to carry the resistance gene found in Richland variety. The other mutant genes that conditioned resistance to races 7 and 7A were not tested against the Richland gene, but the pathologic data would indicate that a majority of the mutations for stem rust resistance were similar to the Richland gene. The reaction type of the mutant strains that were resistant to race 8 indicates that this mutation may be similar to or the same as the White Tartar gene. To date, however, crosses that would test this hypothesis have not been made. It appears that a third mutation conditioning stem rust resistance may have been obtained from the irradiated material. Line A746-2-7 possesses either 1 or 2 genes that condition resistance to races 7 and 8 but not to races 6 and 7A. There is no combination of known genes for stem rust resistance that would give the reaction shown by this line. It would be very encouraging if this line should prove to contain a "new" gene for stem rust resistance, for the search for more genes conditioning resistance to the oat stem rust fungus has been rather futile.

Second, no mutation conditioning resistance to race 6 was obtained from the irradiated material. It was hoped that such a mutation would be found, since only 1 gene producing resistance to this race is now available to plant breeders. This gene, known as the Canadian factor, gives a resistant reaction at normal temperatures, but it induces a susceptible reaction when plants possessing it are grown at 85°F or higher. For these reasons considerable effort is being exerted to find a "new" gene conditioning resistance to race 6.

The Richland and White Tartar genes, which condition resistance to races 7 and 7A and to race 8, respectively, were found by Smith (8) to be allelomorphic, a condition that makes it impossible to include both genes in a pure-line variety of oats.[5] Pathologic and limited genetic data indicate that the mutations for stem rust resistance obtained in this study probably are similar to the Richland and White Tartar genes; therefore, this locus appears to be especially mutable. The mutations did not result from a change of the White Tartar to the Richland gene, or *vice versa*, but both were derived from the allelomorph that conditioned susceptibility in Huron variety to races 7, 7A, and 8 of the oat stem rust pathogen. It will be interesting to study these strains cytologically to determine if some chromosomal rearrangement is taking place to cause the apparent mutations for stem rust resistance.

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[5] F. K. S. Koo has recently isolated, from a rather involved cross, an oat strain that has the Richland and White Tartar genes located on the same chromosome only a small number of cross-over units apart. This probably resulted from an unequal crossing over between homologous chromosomes on which these 2 genes were located (personal correspondence).

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EFFECTS OF TEMPERATURE ON MULTIPLICATION OF POTATO VIRUS X IN NICOTIANA SPECIES [1]

Glenn S. Pound and Katie Helms

SUMMARY

Multiplication of a clear ring strain of potato virus X in *Nicotiana glutinosa* and *N. tabacum* was studied in plants grown at air and soil temperatures of 16°, 20°, 24°, and 28°C. Patterns of virus concentration in relation to air temperatures showed seasonal variations when measured by ultraviolet-absorption assays, but not when measured by local-lesion assays. In winter, virus nucleoprotein concentration as measured by optical density was closely correlated with symptoms; it was greatest at 16° and decreased as temperature increased. When measured by local-lesion assay, virus concentration was low at 16° and usually reached a maximum at 24°, but in some assays maxima were at 20° or 28°, with lesion numbers at 20° and 28° differing little from those at 24°. Data from optical-density assays and virus infectivity assays were more closely associated in spring, when maximum virus protein tended to be at 20°, than in winter. They were more closely associated in *N. tabacum* than in *N. glutinosa*. Symptoms were more severe and virus concentration was greater in *N. glutinosa* than in *N. tabacum*. The reduction in fresh weight of infected plants relative to healthy plants was greater in spring than in winter and greater in *N. glutinosa* than in *N. tabacum*. Effects of soil temperature on symptom expression and virus concentration were similar to but less marked than those of air temperature.

The importance of air temperature in relation to development of symptoms of virus X in potato was first recognized by Johnson (6) in 1922. Under conditions favoring host growth, he found the optimum temperature for disease development to be between 14° and 18°C. Symptoms were completely inhibited within a period of 1–2 weeks at temperatures of 24–25°. Further studies in 1925 by Goss and Peltier (4) and in 1926 by Tompkins (16) indicated that although soil temperature and light were probably factors influencing symptom severity, air temperature was the most important environmental factor. No attempts were made to determine if virus concentration in plants was related to symptom severity.

Recent studies of effects of temperature on multiplication of the viruses of cauliflower mosaic (13), turnip mosaic (12, 13, 14), cucumber mosaic (3), and tobacco mosaic (1) have shown that virus concentration generally is directly correlated with symptom severity. This paper presents data of studies on the effects of air and soil temperature on multiplication of virus X in *Nicotiana glutinosa* L. and *N. tabacum* L. var. *Havana* 38.

Relative virus concentrations in sap of plants grown at 16°, 20°, 24°, and 28° were assayed by an ultraviolet-absorption technique and also by a local-lesion technique. The first of these methods measures extractable nucleoprotein that is presumably virus nucleoprotein which may consist of noninfective as well as infective virus particles, whereas the second method measures only infective virus.

MATERIALS AND METHODS.—The clear ring strain of virus X described by Ladeburg, Larson, and Walker (8) was used in all experiments. It was maintained in *N. glutinosa* in the greenhouse as well as in desiccated tissue. When held after inoculation at an air temperature of 16°, hosts used for virus mutiplication gave a necrotic reaction on inoculated leaves, followed by systemic symptoms. Lesions on *N. glutinosa* were sufficiently discrete for this host to be used in bioassays (8).

Plants for local-lesion assays were grown in soil in 4-in. clay pots at 22°C. Plants for systemic multiplication of the virus were grown in white quartz sand in 7-in. glazed earthenware crocks and were watered with a balanced nutrient solution (5). Thirty-two crocks, each containing 5 plants, were used in each experiment. No artificial lighting was used during winter or summer.

In the air-temperature studies, plants were held at 1 temperature (usually 24°), until they had developed 4 or 5 leaves. Plants in 16 of the 32 crocks were then dusted with 600-mesh Carborundum by means of a powder blower attached to a compressed-air line. Two leaves of each plant were mechanically inoculated (by means of a cheesecloth pad) with a raw juice extract from young infected leaves of *N. glutinosa*. Immediately after inoculation, the leaves were rinsed with water. Four crocks containing inoculated plants and 4 containing control plants were placed in each of 4 houses kept at air temperatures of 16°, 20°, 24°, and 28°.

In the soil-temperature studies, 8 crocks were placed immediately after transplanting in each of 4 soil-temperature tanks kept at 16°, 20°, 24°, and 28°. The air temperature was maintained at 22°. When plants grown at 16° had produced 3 or 4 well-developed leaves, plants in 4 crocks at each temperature were

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inoculated as described for the air-temperature experiments.

At 4 successive weekly intervals following inoculation, inoculated plants in 1 crock and healthy plants in 1 crock from each treatment were harvested by cutting plants at sand level, and all harvested tissue was weighed and frozen.

Both local-lesion and spectrophotometric assays were made from sap samples obtained from the frozen tissue. When the quantity of tissue was small, it was ground with a mortar and pestle. Otherwise it was passed through a food chopper or juice extractor. The pulp or sap extract was filtered through 2 layers of cheesecloth. The filtered sap was kept in a refrigerator at all times except when handled.

For local-lesion assays, sap samples from plants grown at the 4 temperatures were compared by means of an incomplete-block design in which there were 9 half-leaf replicates of each treatment (12). The day prior to inoculation, assay plants were selected for uniformity and all but 3 successive leaves of medium age were removed. Leaves were inoculated with sap diluted 1:400 with distilled water. They were subsequently rinsed with distilled water, and the plants were placed in a room maintained at 16°. Local lesions were counted after 6–8 days. Tests of significance between any 2 treatments were made by an analysis of variance. To simplify presentation, the treatment averages shown in the tables are the over-all averages of 9 replicates. They indicate virus concentration gradients, but do not indicate true differences among treatments because of variation among individual leaves of assay plants. This variation was taken into account in the analyses in which half-leaf comparisons were made.

Spectrophotometric analyses were made on partially purified extracts from virus-infected plants and from healthy plants. Sap samples of at least 4 ml were diluted with an equal volume of 0.1M phosphate buffer of pH 7.0. Coarse matter was removed by 2 or 3 successive 20-minute centrifugation cycles at 6000 rpm (approx. $2400 \times g$). Five-ml aliquots of the final supernatant, together with sufficient buffer to fill the tubes to capacity, were centrifuged for 90 minutes at 35,000 rpm (approx. $80,000 \times g$) in a Spinco Model L refrigerated ultracentrifuge. The supernatants were discarded and the tubes thoroughly drained. Pellets were homogenized with 5 ml buffer by an electrically-driven stirrer fitted with a Teflon homogenizer and run at low speed. These extracts were centrifuged for 30 minutes at 6000 rpm and optical-density readings were made on the diluted or undiluted supernatants by means of a Beckman Model DU spectrophotometer set at 265 mμ, the wave length of the maximum absorption for the purified virus (10).

When 1 cycle of low and high speed was insufficient to rid the solutions of green coloring matter, these impurities were precipitated by an additional cycle of high and low speed, or by acidifying the solutions to pH 5 with 10 per cent acetic acid. Some loss of virus occurred during both procedures, but it was less when the latter method was used. In Figure 1 are shown ultraviolet-absorption curves of a virus extract obtained after 1 and 2 cycles of high- and low-speed centrifugation. It can be seen that loss of virus by a second cycle of centrifugation was significant. Readings for virus concentration were made as soon as possible after centrifugation because preparations aggregate on standing (2, 9). The concentration of virus in the virus extract was considered proportional to the difference between the optical-density value of the virus extract and that of the extract from corresponding healthy plants. Data were obtained directly by using the fractions from healthy plants as blanks for optical-density readings of the virus extracts. Similar methods have been used recently by Schneider (15) and by Kirkpatrick and Lindner (7). The assumption is made that virus infection promotes neither formation nor breakdown of substances (other than virus) that absorb at the wave length absorbed by purified virus.

Ultraviolet-absorption values of virus fractions from infected plants, taken at 5-mμ intervals in the range of 230–300 mμ, minus the corresponding values of fractions from noninfected plants receiving the same treatments were as shown in Figure 1 and were characteristic of those of the purified virus (10). It was considered that the method would give information on the relative amounts of virus in infected plants

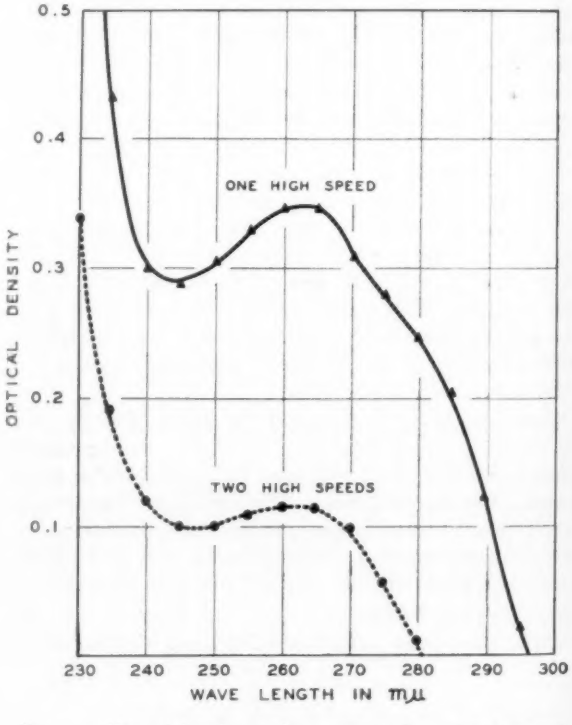

FIG. 1. Ultraviolet-absorption curves of a virus extract obtained by 1 and 2 cycles of low- and high-speed centrifugation. Note the loss of virus in the second cycle.

grown at different temperatures where differences were large, even though it might not be sufficiently accurate to do so where differences were small. In most assays when optical-density readings of extracts were made using phosphate buffer as a blank, values for extracts from noninoculated plants followed the same pattern as values for extracts of inoculated plants in relation to temperature. In experiments with *N. glutinosa*, values for extracts of inoculated plants were usually between 2 and 5 times greater than those for extracts of noninoculated plants. For example, in an air temperature experiment, an assay made 14 days after inoculation showed optical-density values for extracts of inoculated plants grown at 16°, 20°, 24°, and 28° to be 0.625, 0.350, 0.215, and 0.125, respectively, and values for noninoculated plants to be 0.115, 0.075, 0.052, and 0.020, respectively. In experiments with *N. tabacum*, values for extracts of inoculated plants were usually not more than twice those of values for extracts of noninoculated plants. The data suggested that the method probably resulted in an under-estimation of virus rather than an over-estimation, since in occasional assays (data of which are not presented) the optical-density value for the control extract was greater than or equal to that for the virus extract, which when tested proved to be infectious. Apparently the concentration of extracted normal nucleoprotein from healthy plants was sometimes greater than that of normal nucleoprotein plus virus nucleoprotein of virus-infected plants.

EXPERIMENTAL RESULTS.—*Effect of air temperature on symptoms.*—Temperature had a pronounced effect on growth of inoculated and healthy plants. During the 4 weeks of each experiment, marked stunting occurred at 16° and maximum growth was at 24° or at 28°.

In an experiment with *N. tabacum* made in January, the mean fresh weight per plant, averaged over 4 harvests, of plants grown at 16°, 20°, 24°, and 28° was 5.1, 8.1, 12.6, and 12.0 g, respectively, for inoculated plants and 6.4, 9.0, 12.5, and 13.0 g, respectively, for noninoculated plants. In an experiment in March the means were 7.7, 8.5, 11.1, and 17.7 g, respectively, for inoculated plants and 12.2, 11.4, 14.2, and 16.5 g, respectively, for noninoculated plants.

Symptoms in spring were less severe than in winter. Following inoculation, local lesions developed most slowly at 16°. They became clearly defined after 5 to 7 days and soon caused considerable necrosis. Local lesions on plants grown at 20° were similar to those at 24°. They constituted a distinct ring-spot mottle and were not as severe as at 16°. Local lesions were mildest at 28° (Figure 2). One week after inoculation, plants grown at 24° and 28° showed a conspicuous systemic mottle, whereas at 20° veinclearing was the only systemic symptom. Two weeks after inoculation, a mild mottle had developed on plants held at 16° and 20°, and 1 or 2 weeks later systemic symptoms were more severe than those of plants at 24° or 28°. Thus at low temperatures, both local lesions and systemic symptoms developed more slowly than at higher temperatures, but eventually became more severe.

Virus movement into younger leaves was slow. Tip leaves were always symptomless, slightly older leaves developed a mottle at their tips and still older leaves showed general systemic symptoms. There were more symptomless young leaves on plants grown at 24° or 28° than on those grown at 16° or 20°.

In an experiment with *N. glutinosa* made in December, the mean fresh weight per plant, averaged over 4 harvests, of plants grown at 16°, 20°, 24°, and 28°

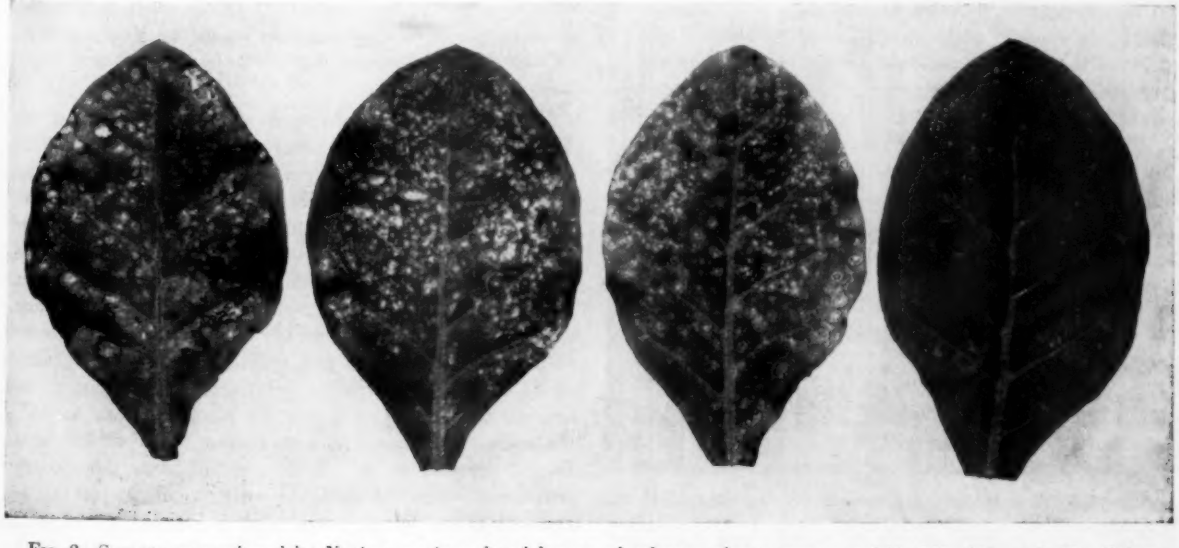

FIG. 2. Symptoms produced by X virus on inoculated leaves of tobacco plants grown at 16°, 20°, 24°, and 28° (left to right) after inoculation.

was 2.0, 2.9, 3.1, and 3.7 g, respectively, for inoculated plants and 4.2, 5.0, 4.9, and 4.7 g, respectively, for noninoculated plants. In an experiment in March the means were 3.4, 4.7, 9.9, and 11.7 g, respectively, for inoculated plants and 10.8, 14.0, 18.1, and 14.3 g, respectively, for noninoculated plants.

Symptoms on *N. glutinosa* were more severe than on *N. tabacum*. The reduction in fresh weight of infected plants relative to healthy plants was greater in spring than in winter (Figure 3). Symptom development followed the same pattern as in *N. tabacum* except that the virus moved more rapidly into the younger leaves.

Effect of air temperature on virus concentration.—1) *N. tabacum.*—In 3 experiments made between January and April, both local-lesion and optical-density data were obtained for virus concentration, and in 2 experiments only optical-density data were obtained. Optical-density readings suggested that seasonal factors affect the relative concentration of virus at different temperatures. In an experiemnt in January, the greatest virus concentration as measured by optical density 14, 21, and 28 days after inoculation was at 16°, whereas in experiments begun in March (Table 1), the maximum virus concentration was at 20°. Virus concentration at 28° was usually lower than at any other temperature. Data for *N. tabacum* in Table 1 are representative of those obtained in experiments made in March, although in the third assay of 1 experiment made in March, the maximum virus concentration was unexpectedly at 24°. In general, the data indicate a virus multiplication gradient that decreases from low to high temperatures, thus showing a direct correlation with symptom expression.

The above pattern of virus concentration was not always evident 7 days after inoculation; in winter and spring experiments, optical-density measurements showed the highest virus titre to be at 20°, 24°, or 28°. This irregularity was associated with the early development of systemic symptoms, which appeared first at the higher temperatures and later at the lower temperatures.

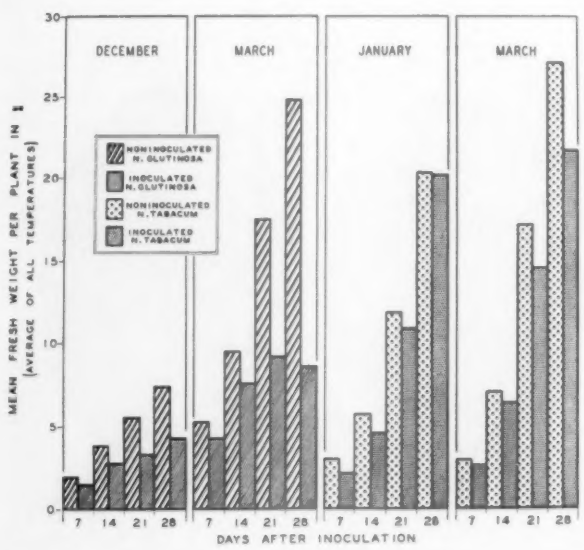

FIG. 3. Comparative growth of healthy and inoculated plants of *N. glutinosa* and *N. tabacum* in winter and spring experiments.

TABLE 1.—*Optical-density and local-lesion assays of virus X in extracts from plants of Nicotiana tabacum and N. glutinosa grown at various air temperatures*

		Optical-density and local-lesion values for virus concentration[a]			
		N. tabacum[b]		N. glutinosa[b]	
Days after inoculation	Temperature (°C)	Optical density	Average no. local lesions per half leaf[c]	Optical density	Average no. local lesions per half leaf[c]
7	16	0.125	11.2	0.280	2.9
	20	0.195	29.9	0.100	18.6
	24	0.110	30.0	0.240	39.9
	28	0.015	14.9	0.290	17.1
14	16	0.020	36.3	0.510	4.9
	20	0.185	103.2	0.285	81.8
	24	0.180	128.6	0.163	81.0
	28	0.105	64.5	0.110	80.6
21	16	0.128	99.2	1.050	62.2
	20	0.140	163.3	0.700	201.6
	24	0.053	39.5[d]	0.302	225.0
	28	0.049	93.1	0.185	140.0
28	16	0.343	149.2	1.100	88.0
	20	0.562	198.1	0.590	186.8
	24	0.250	113.5	0.200	193.1
	28	0.118	92.1	0.115	171.7

[a] For each host, optical-density and local-lesion data are from different experiments.
[b] The 2 experiments with *N. glutinosa* were in December; those with *N. tabacum* were in March.
[c] Average of 9 half-leaf replications.
[d] This value was unaccountably low.

Maximum virus concentration as measured by optical density was not correlated with maximum growth, which was consistently at 28° in March and at 24° or 28° in January for both inoculated and noninoculated plants.

Representative local-lesion data are included in Table 1. In 11 of 16 assays the number of lesions from extracts of plants grown at 16° was significantly less than that from extracts of plants grown at 1 or more of the other temperatures. In 6 assays the lesion number from extracts of plants grown at 28° was significantly less than from extracts of plants grown at 24° or 20°. In only 2 assays was there a significant difference between the number of lesions from extracts of plants grown at 20° and from those of plants grown at 24°. To summarize, a relatively small number of lesions developed from sap of plants grown at 16°, the number increased to a maximum at 20° or 24°, and then decreased at 28°. Lesion counts were not closely correlated with the optical-density readings for virus concentration, which were highest at 16° or at 20°. Lesion counts showed some correlation with fresh weight in that they were lowest at 16°. The number

of local lesions at 28° was always less than at 20° or 24°, whereas the fresh-weight yield at 28° was sometimes greater than at 24°. It should be noted that growth differences at 24° and 28° were usually small.

2) *N. glutinosa.*—Both optical-density and local-lesion data were obtained in 3 of 4 experiments. In the fourth experiment only optical-density data were obtained. Optical-density values for virus concentration were similar to those obtained with extracts of *N. tabacum*. In December, highest virus concentration was consistently at 16° at intervals of 14, 21, and 28 days after inoculation (Table 1), whereas in March it was at either 16° or 20°. One week after inoculation the virus concentration pattern was irregular, as was the case with *N. tabacum*.

In 14 of 15 local-lesion assays, the number of lesions produced by extracts of plants grown at 16° was significantly less than that by extracts of plants grown at 1 or more of the higher temperatures. Infectivity increased with increased temperature and either reached a maximum at 24° and decreased at 28°, as in the experiment for which data are given in Table 1, or was highest at 28°. Differences between lesion numbers from sap of plants grown at 24° and 28° usually were not significant. The local-lesion pattern differed from the optical-density pattern, which showed that virus concentration was highest at 16° or 20°. Infectivity was closely correlated with fresh weight, which was lowest at 16° and highest at 24° or 28°.

Effect of soil temperature on symptoms.—The growth patterns in relation to soil temperature were similar to those for air temperature. Least growth was at a soil temperature of 16° and greatest growth at 24° or 28°. In an experiment made in November with *N. glutinosa* for which data on virus concentration are shown in Table 2, the mean fresh-weight yield per plant, averaged over 4 harvests, of plants grown at 16°, 20°, 24°, and 28° was 1.9, 3.0, 4.0, and 3.6 g, respectively, for inoculated plants and 4.7, 5.1, 5.5, and 5.6 g, respectively, for noninoculated plants.

TABLE 2.—*Optical-density assays of virus X in extracts from plants of Nicotiana glutinosa grown at various soil temperatures*[a]

Days after inoculation	Temperature (°C)	Optical-density values
7	16	0.700
	20	0.500
	24	0.468
	28	0.582
14	16	0.890
	20	0.330
	24	0.255
	28	0.190
21	16	0.465
	20	0.790[b]
	24	0.315
	28	0.295
28	16	0.345
	20	0.310
	24	0.285
	28	0.180

[a] The experiment was made in November.
[b] This value was unexpectedly high.

One week after inoculation, plants of *N. glutinosa* grown at 16° showed necrotic ring spots and specks on inoculated leaves. At 20°, there was less necrosis, and at 24° and 28° inoculated leaves developed only a ring-spot pattern. Systemic mottling was at first more severe at the higher temperatures, but 14 days after inoculation symptoms were the most severe at 16°. Although stunting decreased with increase in temperature, there was at first little difference in the systemic mottle of plants grown at 20°, 24°, and 28°. Three or 4 weeks after inoculation, the systemic symptoms of plants at 28° were milder than those of plants grown at lower temperatures.

As in the air-temperature experiments, symptoms on *N. tabacum* were not as severe as those on *N. glutinosa*, and other than during the first 7–14 days after inoculation, tip leaves showed no systemic mottle.

Effect of soil temperature on virus concentration.—1) *N. glutinosa.*—In 3 experiments made between October and December, effects of differential soil temperatures on virus concentration and on symptoms were less marked than but essentially the same as those in air-temperature studies made at the same time of the year. Optical-density readings for virus concentration 14, 21, and 28 days after inoculation (Table 2) tended to decrease with increases in temperature. Exceptions occurred in the third assay of the experiment shown in Table 2, where the maximum was at 20°, and in the fourth assay of another experiment, in which the maximum was at 24°. In 10 of the 11 assays, virus concentration at 16° was greater than at 28°. Differences in virus concentration as measured by local lesions were small and usually not significant.

2) *N. tabacum.*—Experiments were made in April and May. Differences in virus concentrations at the various temperatures in April were small, but virus concentrations by both assays were highest at 24° at 21 and 28 days after inoculation. As a result of high air temperatures in May, virus concentrations obtained 21 and 28 days after inoculation were almost the same for each temperature. As was the case with *N. glutinosa*, symptom development and multiplication of virus X in *N. tabacum* were affected more by air temperature than by soil temperature. Virus-infected *N. tabacum*, however, showed a smaller response to variations of soil and air temperature than did virus-infected *N. glutinosa*.

Soil temperature affected symptom expression to a lesser degree than air temperature. Similar data were obtained by Cheo and Pound (3) in studies of cucumber mosaic virus and by Pound and Weathers (14) in studies of turnip mosaic virus.

DISCUSSION.—In the greenhouse, potato virus X characteristically produces mild symptoms in summer and severe symptoms in winter. Early work indicated that symptom expression is correlated with air and soil temperature. Studies presented in this paper on

the multiplication of a clear ring strain of virus X in *N. tabacum* and *N. glutinosa* support this work and show that variations in symptom expression with temperature are correlated with changes in virus concentration. It is surmised that the same situation would exist in potato.

There was no indication of a cycle of virus activity associated with temperature, as found by Cheo and Pound (3) in tip-leaf assays of tobacco infected with cucumber mosaic virus and by Bancroft and Pound (1) in similar assays of tobacco plants infected with tobacco mosaic virus. This may be associated with the fact that following inoculation of the lower leaves of the host, both cucumber mosaic virus and tobacco mosaic virus move rapidly to the tip leaves, whereas virus X moves relatively slowly. In an experiment in which comparisons were made of local lesions obtained with sap of tip leaves of *N. tabacum* and sap of whole plants, virus concentrations in tip leaves were between 1/20 and 1/100 of that in samples from whole plants.

Symptoms were consistently more severe, and reduction in fresh-weight yield of infected plants was greater, in *N. glutinosa* than in *N. tabacum*. When similar sap extracts were compared at the same interval after inoculation, virus concentration was always greater in *N. glutinosa* than in *N. tabacum*. Loring and Wyckoff (11) similarly found a greater concentration of virus X protein in *N. glutinosa* than in Turkish tobacco. Johnson (6) found symptoms of potato plants infected by virus X to be completely inhibited at 24° or 28° after 1 or 2 weeks. The tobacco plants in experiments in this paper showed reduced symptom severity at air and soil temperatures of 24° and 28°, but symptoms were never completely inhibited.

Data indicate that the multiplication pattern of virus X is influenced by host species as well as by temperature and season. As symptom expression was affected by season when temperature was controlled, it is thought that light intensity and day length may be the seasonal factors involved. Further work with these environmental factors may clarify the data obtained on differences between concentration patterns of virus as measured by optical density and by local-lesion assay. For example, in winter the highest concentration of virus nucleoprotein and the lowest concentration of infective virus developed at 16°. In spring the 2 were more closely associated, the highest concentration of virus nucleoprotein being at 20° and the highest concentration of infective virus at 20° or 24°. The highest concentration of virus nucleoprotein was thus associated with reduced growth and severe symptoms, whereas the highest concentration of infective virus was associated with more vigorous host growth.

Most published work shows a correlation between concentration of virus nucleoprotein as measured by optical density and concentration of infective virus as measured by local-lesion assay. Weathers and Pound (17) found, however, that tobacco plants given 3 times the normal concentration of a balanced nutrient solution formed a higher concentration of virus as measured by optical density than could be accounted for by local-lesion assay. They concluded that noninfective virus was formed at the high nitrogen level.

There is considerable evidence to indicate that all virus particles formed in an infected host can not be assumed to be identical (even though they appear homogeneous by the usual physico-chemical tests), nor can it be assumed that all the virus particles are equally infective. At present there is no way of measuring the proportion of noninfective to infective particles or indeed the number of particles required for infection. The following suggestions are submitted as possible explanations for the lack of correlation of the data obtained for concentration of virus X when measured by the optical-density and local-lesion techniques. First, the proportion of infective to noninfective virus particles (or nonlesion forming virus), may vary under different environmental conditions. At 16° and to a lesser extent at 20°, host plants may produce a greater proportion of noninfective to infective particles than at 24°. If this is true, then likewise it is possible that the proportion of noninfective to infective virus was greater in winter than in spring and greater in *N. glutinosa* than in *N. tabacum*. Secondly, the proportion of infective virus formed may be approximately the same under all 4 temperature conditions, but a third substance, an inhibitor that decreases the infectivity of the sap extract, may be formed at low temperatures. Weathers and Pound (17) attributed reduced infectivity of sap of mosaic-infected tobacco plants grown at high levels of nitrogen to formation of an inhibitor. Thirdly, the possibility exists that the differences were primarily due to the techniques used. The method by which optical-density values for virus concentration were obtained was based, as stated earlier, on differences in optical-density values of partially-purified extracts of healthy and virus-infected plants, the assumption being made that infection promotes neither the formation nor the breakdown of substances (other than virus) that absorb at the wave length of the purified virus. In regard to the local-lesion assay, it is possible that this method of assay may be less accurate for virus X than it is for some other host-virus combinations. This is because virus X particles show considerable aggregation in solution, and therefore the number of virus particles which together result in the formation of a single local lesion on *N. glutinosa* may vary greatly. The fact that the local-lesion reaction is dependent on growing assay plants after inoculation for 6 to 8 days at a relatively low air temperature may also result in inaccuracies. It seems reasonable to assume that in a slowly-developing disease reaction many potential infection foci may not develop.

As tests for the presence of an inhibitor were not made, and alternative methods of obtaining virus extracts were not used, the authors do not know which, if any, of these possible explanations is applicable.

With many viruses however, severity of symptoms of a given virus has been associated with relatively high virus concentrations as measured by the local-lesion assay. Since severe symptoms of virus X and relatively low numbers of local lesions are associated with low temperatures, it seems likely that with this virus an inhibitor that reduces lesion formation is formed at low temperature, and hence optical-density readings, which are clearly associated with symptom severity, give a better measure of total virus concentration than does the local-lesion assay.

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SYMPTOMS OF BUD-BLIGHT OF SOYBEANS CAUSED BY THE TOBACCO- AND TOMATO-RINGSPOT VIRUSES [1]

Robert P. Kahn and Frances M. Latterell

SUMMARY

Soybean plants infected with the tobacco-ringspot virus may be characterized by several important symptoms that apparently have not been reported in the literature. These symptoms include 1) stunting, 2) proliferation of floral buds and abortion of pods, 3) proliferation of trifoliate leaves, 4) abnormally dark green leaves, and 5) swollen nodes.

The tomato-ringspot virus induced typical bud-blight symptoms in greenhouse- and field-grown soybean plants. This is believed to be the first report of this virus as a causal agent of soybean bud-blight.

The tobacco-ringspot virus was discovered to be the causal agent of the bud-blight disease of soybeans by Allington in 1946[2]. The symptoms of the disease as described by Allington were as follows: 1) characteristic curving of terminal buds, 2) bronzed appearance of young leaves, 3) unusually prominent and darkened pubescence on stem tip, 4) necrosis and brittleness of the stem tip, 5) reddish-brown discoloration in the pith of stems and branches, 6) failure of the plants to mature, 7) withering and abscission of pod clusters, and 8) blotching on pods with reduction in seed quality.

The symptoms of the disease as reported by Hildebrand and Koch in 1947[3] included 1) characteristic distortion, brittleness, and necrosis of the shoot tip; 2) yellow discoloration of leaves with a tendency toward rugoseness, rolling, vein-clearing, necrotic stippling, and bronzing; 3) blighting of buds and blossoms with a marked reduction in yield and size of pods; and 4) frequent discoloration of pith.

During the past 3 years the writers have observed additional characteristic symptoms in greenhouse- and field-grown soybeans inoculated with this virus. The tomato-ringspot virus (tobacco-ringspot virus No. 2) also was found to induce typical bud-blight symptoms in soybeans, an observation which insofar as the authors are aware has not been reported. It is the purpose of this paper to describe more fully the symptoms of bud-blight as caused by the tobacco-ringspot virus and to report that typical bud-blight symptoms can be induced by inoculating soybeans with the tomato-ringspot virus.

MATERIALS AND METHODS.—The virus cultures used in these experiments were obtained from Dr. W. B. Allington, who supplied the tobacco-ringspot virus, and from Dr. W. C. Price, who furnished the tomato-ringspot virus. Since both viruses were found to produce essentially the same systemic symptoms in soybeans and tobacco and the same type of local lesions in cowpeas and Pinto beans, it was necessary to check the identity of these virus cultures by some other means than host range or symptomology. The viruses were differentiated, therefore, by cross-protection experiments as described by Tall et al.[4] In these tests, rooted stem tips from tobacco plants recovered from tobacco ringspot were protected against reinoculation with tobacco-ringspot virus but not tomato-ringspot virus. Similarly, rooted stem tips from plants recovered from tomato ringspot were protected against reinoculation with tomato-ringspot virus but not against tobacco-ringspot virus. The 2 viruses were also distinguished on the basis of particle size and shape as previously reported in electron microscope investigations.[5, 6]

Inoculations were accomplished by rubbing Celite- or Carborundum-dusted leaves of soybean plants of the Lincoln and Bansei varieties with gauze pads dipped in the inoculum. The inocula were prepared from both fresh and frozen infected tobacco and soybean leaves, and also from desiccated tobacco and bean leaves by extraction with 0.1M potassium phosphate buffer (pH 7). Most of the inoculations were made when the plants were in the primary leaf stage although in some instances older plants were used. In 1 experiment, 4 dilutions of inoculum for each virus were used and the symptoms compared. Symptoms were observed both in summer and winter months for greenhouse-grown plants. During the summer 1 set of plants was incubated in a controlled temperature greenhouse between 20 and 26°C while another set was incubated at the usual summer temperature fluctuations in the greenhouse, between 21 and 35°C.

UNREPORTED SYMPTOMS OF BUD-BLIGHT OF SOYBEANS CAUSED BY TOBACCO-RINGSPOT VIRUS.—Three years of observations of greenhouse- and field-grown soybeans

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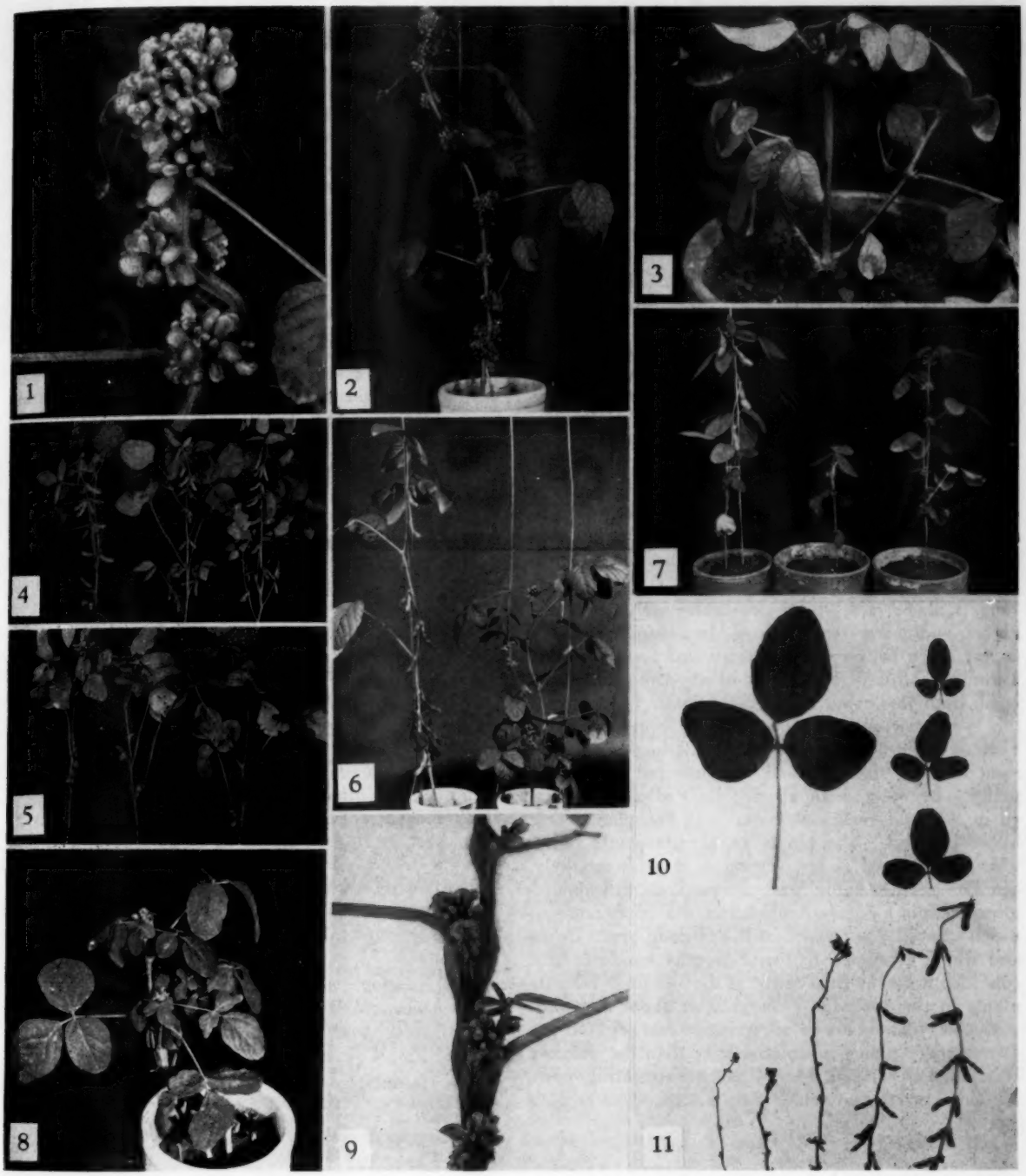

FIG. 1–11. Soybean plants infected with tobacco- or tomato-ringspot virus, and virus-free soybeans.—Fig. 1, 2. Proliferation of buds and abortion of pods caused by tobacco-ringspot virus.—Fig. 3. Swollen nodes caused by tobacco-ringspot virus, 6-month-old plant.—Fig. 4. Virus-free field-grown soybeans.—Fig. 5. Field-grown soybeans infected with tobacco-ringspot virus.—Fig. 6. Left, virus-free plant; right, plant infected with tobacco-ringspot virus, showing stunting, bud and leaf proliferation, and pod abortion.—Fig. 7. Relative stunting by the 2 viruses. Left, virus-free plant; center, infected with tobacco-ringspot virus; right, infected with tomato-ringspot virus.—Fig. 8. Swollen nodes, shortened internodes, and leaf proliferation caused by tobacco-ringspot virus.—Fig. 9. Bud proliferation and swollen nodes caused by tobacco-ringspot virus.—Fig. 10. Left, leaf from virus-free plant; right, leaves from clusters of proliferated leaves from plants infected with tobacco-ringspot virus.—Fig. 11. Soybean stems collected in the field at the end of the growing season. Left, 2 stems from plants infected with tobacco-ringspot virus; center, stem from plant infected with tomato-ringspot virus; right, 2 stems from virus-free plants. (Photographs taken by George A. Evans.)

indicated that infected plants may be characterized by several important symptoms which have not been reported in the literature. These symptoms include 1) stunting, 2) proliferation of floral buds and abortion of pods, 3) proliferation of trifoliate leaves, 4) abnormally dark green leaves, and 5) swollen nodes.

The stunting symptoms due to shortened internodes or to a reduced number of nodes (Fig. 6, 7, 11) were not usually evident when inoculated plants were incubated at the higher temperatures. For instance, stunting was not apparent in the 1954 field tests when a 3-week period of abnormally high daytime temperatures occurred immediately following inoculations. In 1951, 1952, and 1953, however, when no extremely hot periods followed inoculation, the stunting symptoms were pronounced. In the greenhouse, similar results were obtained. Inoculated plants incubated in the controlled temperature greenhouse at below 26° usually were stunted when compared with healthy plants grown at the same low temperature. On the other hand, inoculated plants incubated at 24–35° frequently attained the same height as the noninoculated controls although they showed other bud-blight symptoms. Stunting was not evident when plants were more than 5 weeks old at the time of inoculation.

The excessive proliferation of leaves and floral buds (Fig. 1, 2, 6, 7, 8, 9, 11) was observed at both the higher and lower temperatures, in plants inoculated at any stage between the primary leaf and flowering. Terminal and axillary clusters of abortive pods developed from the proliferated floral buds, but pods of this type grew only to about 1 cm in length (Fig. 1). Figures 4 and 5 illustrate the contrast in pod development between virus-free and virus-infected field-grown plants. Leaves and the aborted pods of plants infected in the field remained green until the plants were killed by frost. The plants apparently would otherwise have existed in the vegetative stage indefinitely. Similar growth habit was observed in greenhouse-grown plants. Figure 3 illustrates the appearance of a 6-month-old plant which still had dark green leaves and which continued to live 2 months longer in this condition until the stem rotted at the soil line. Healthy plants in the greenhouse matured at about 3 months with abscission of leaves occurring soon after seed set. Occasionally plants inoculated later than the primary leaf stage developed 1 or 2 normal-appearing pods, but these usually contained only 1 seed, whereas pods on virus-free plants contained 3 or 4 seeds. The proliferation of leaves (Fig. 6, 8) occurred to the extent of 5–15 more trifoliate leaves per virus-infected plant than on a healthy plant. Such leaves were usually ⅓ to ½ the size of healthy leaves, and were darker green than the normal leaves first initiated from the nodes of virus-infected plants (Fig. 10).

SYMPTOMS OF BUD-BLIGHT OF SOYBEANS CAUSED BY THE TOMATO-RINGSPOT VIRUS.—The symptoms of bud-blight of soybeans caused by the tomato-ringspot virus were essentially the same as those described for the tobacco-ringspot virus. Two of the symptoms, i.e. shortened internodes and failure of pods to mature, were somewhat less severe with the tomato-ringspot virus even at the lower temperatures. Thus, while both viruses caused stunting at the lower temperatures, the stunting was less pronounced in plants infected with the tomato-ringspot virus (Fig. 7, 11). Pod production was repressed by both viruses, but to a lesser degree by tomato-ringspot virus. Plants infected with tobacco-ringspot virus rarely produced more than 2 mature pods, whereas 5–7 pods often matured on plants infected with tomato-ringspot virus. Approximately 20–25 pods usually matured on healthy plants in the greenhouse. These differences in degree of symptom expression were not apparently associated with the concentration of the 2 viruses in their respective inocula since the differences in severity were evident at all 4 concentrations employed (10-fold dilutions, from 1:1 to 1:1000).

Neither the source of the inoculum nor the concentration of virus in the inoculum within the range evaluated was a factor in affecting symptom expression in soybeans inoculated with either of the ringspot viruses. Symptoms of infection by both viruses were essentially the same whether the inoculum was extracted from fresh or frozen tobacco leaves or from desiccated tobacco or bean leaves. The concentration of virus in the inoculum within the range evaluated was apparently not an important factor in symptom expression since the symptoms reported herein were observed at all dilutions.

The symptoms as described were observed in both the Lincoln and Bansei varieties of soybeans as a result of greenhouse and field inoculations.

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EFFECTS OF CALCIUM SULFAMATE AND SODIUM SULFANILATE ON SMALL GRAINS AND ON STEM RUST DEVELOPMENT[1]

Aristeo Acosta C. and J. E. Livingston[2]

SUMMARY

Two applications of calcium sulfamate to plants at the rate of either 9 or 12 lb. per acre, the first when approximately 50 per cent of the plants had pollinated and the second 1 week later, markedly reduced the germination of wheat and oat seed but only slightly reduced the germination of barley seed. The effect on yield and kernel weights was somewhat variable. The yield of the wheat varieties was increased in 3 out of the 4 treatments and that of oats in 2 of the 4 trials. Stem rust was substantially reduced on each crop.

No injury could be detected from the sodium sulfanilate treatments. Increased yields were obtained with wheat, and significant stem rust control resulted from most of the treatments.

The stage of growth at which single applications of calcium sulfamate were made was related to the resulting injury to spring wheat. Reduced yields were obtained only when applications were made at the tillering stage and reduced germination with applications following pollination.

Applications of sodium sulfanilate 1 week after tillering and 6 days after blooming resulted in a significant increase in yield.

The control of leaf rust (caused by *Puccinia rubigovera* (DC.) Wint. f. sp. *tritici* (Eriks.) Carl.) and stem rust (caused by *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E. Henn.) of small grains with chemotherapeutants has received considerable attention recently, mainly because of the appearance of virulent races of the pathogens to which commercial varieties are susceptible. Livingston (4), Levine (3), Brentzel (1), and Semeniuk (6) have obtained good control with calcium sulfamate, but they also reported injury from the chemical. Livingston (4) obtained moderate control with Acti-dione (3-[2-(3,5-dimethyl-2-oxocyclohexyl]glutarimide) and sodium sulfanilate. The present investigations were undertaken to gain a better understanding of the types of injury that result and the conditions that promote injury to plants treated with either calcium sulfamate or sodium sulfanilate.[3]

MATERIALS AND METHODS.—The spring wheat varieties Centernario, Mida, Rushmore, and Nugget; the spring barley varieties Feebar and Custer; and the oat varieties Nemaha and Kanota were used. All of these except Feebar showed susceptibility to prevalent races of the stem rust organism.

Various concentrations of calcium sulfamate and sodium sulfanilate were applied in water at a rate equivalent to 5 gal. of solution per acre. "Tween 20"[4] was used as a wetting agent.

The spring wheat varieties were planted at the Agronomy Farm near Lincoln, Nebraska, on April 8, 1953, in 10-ft., 4-row plots in which 40 g of seed was used per plot. Data were taken from 8-ft. sections of the 2 center rows. The design used was a modified split-plot, with 3 replications of each of the following treatments: 1) calcium sulfamate 0.45 and 0.60 lb. per quart of water, equivalent to 6 and 9 lb. per acre, respectively; 2) sodium sulfanilate 0.30 and 0.45 lb. per quart of water, equivalent to 6 and 9 lb. per acre, respectively ; and 3) untreated checks. One application of each treatment was made when approximately 50 per cent of the plants had completed pollination on each of 6 plots, and a second was made 1 week later on half of the previously treated plots, resulting in 3 replications with a single application of each treatment and 3 plots with 2 applications.

A similar experiment with the same design and treatments and with 4 replicated plots was made for barley and oats. In the case of barley, the second application was made 4 days after pollination.

An additional experiment with Mida spring wheat was made to determine the effects of applying the chemicals at different stages of growth. Randomized block designs were used with the same type of plots as in the varietal studies. Three replications were used in the experiment involving calcium sulfamate and 2 in the one involving sodium sulfanilate. Aqueous solutions of calcium sulfamate and sodium sulfanilate were applied at the rates of 12 and 9 lb. per acre, respectively. A single application of the chemicals was made at the tillering stage, 1 week after tillering, at the boot stage, at the bloom stage, 6 days after bloom, and 11 days after bloom. The untreated check plots were included at each period of treatment.

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[2] Ingeniero Agronomo, The Rockefeller Foundation, Mexico City, Mexico, and Plant Pathologist, University of Nebraska, Lincoln, Nebraska, respectively.

[3] The authors are indebted to Dr. D. L. Stoddard of E. I. DuPont de Nemours and Co. and Dr. E. G. Jaworski of Monsanto Chemical Co. for supplying calcium sulfamate and sodium sulfanilate, respectively.

[4] Polyoxyethylene sorbitan monolaurate, a non-ionic wetting agent supplied by the Atlas Powder Co.

In all the experiments described the chemicals were applied with a specially designed hand sprayer that operated at a constant pressure permitting an application rate of 5 gal. of solution per acre.

The data recorded for all the experiments included yield in g per plot, germination percentage, weight of 100 kernels in g, total weight per plot in g (straw plus grain), and percentage of infection by the stem rust organism, *P. graminis* f. sp. *tritici*.

From each plot a random sample of 100 kernels was weighed and tested for germination at a temperature of 20–23°C. The samples of wheat and barley remained in the germinator for 7 days and the oats for 10 days. Data on germination were taken on the last day.

Stem rust developed abundantly on the untreated barley and oats and moderately on untreated wheat. Percentage of infection was determined according to the modified Cobb scale.

EXPERIMENTAL RESULTS.—*Effect on stem rust, yield, and kernel weight.*—Calcium sulfamate reduced the percentage of stem rust on wheat, even with a single application of 9 lb. per acre, and markedly reduced stem rust on barley and oats (Table 1), according to expectations in view of previous results (1, 3, 4, 6). Sodium sulfanilate reduced stem rust on the 3 grains but was not as effective as calcium sulfamate.

The effect of calcium sulfamate on yield varied somewhat with the 3 crops. A significant increase with wheat was obtained with a single application of 9 lb. per acre. A significant decrease resulted from 2 applications of the 12-lb. rate on wheat and from all but the single application of 9 lb. per acre on barley. This conforms with Livingston's (4) previous results, which indicated that 2 applications of 15 lb. of calcium sulfamate per acre was the maximum that could be safely used on wheat in the field. This is probably also true with oats since a reduction in yield was indicated with 2 applications of both the 9- and 12-lb. rates.

The barley varieties were more seriously affected by treatment with calcium sulfamate than the wheats and oats, with a significant reduction in yield from 3 of the 4 treatments. This reduction was correlated with a decrease in the weight of individual kernels. On the other hand, with the wheat varieties there appeared to be no relation between the effect of calcium sulfamate on kernel weight and on yield.

None of the sodium sulfanilate treatments caused a reduction in yield or kernel weight. In fact, on the wheats a significant increase in yield and kernel weight was obtained with 3 of the 4 treatments.

Effect on germination.—Two applications of calcium sulfamate brought about a substantial reduction in germination of all the grains tested when applied at either the 9- or 12-lb. rate. A single application did not produce a significant reduction.

Sodium sulfanilate had no effect on germination.

Effect of the chemicals on wheat when applied at different stages of growth.—In the experiment on Mida wheat, a single application of calcium sulfamate caused no increase in yield, regardless of the stage of growth at which it was applied (Table 2), and gave a significant decrease when applied at the tillering stage. Germination was reduced only when the application was made following pollination. Kernel weights increased progressively as applications were made at later stages of growth until the final application when a reduction was recorded. Significant increases over

TABLE 1.—*Various characteristics of small grains treated with different concentrations of calcium sulfamate and sodium sulfanilate in single and split applications*

Characteristic studied	Rate per acre and number of applications of—										
	None	Calcium sulfamate				Sodium sulfanilate				LSD to compare treatment means with checks	
		9 lb.		12 lb.		9 lb.		12 lb.			
		1	2	1	2	1	2	1	2	0.05	0.01
Wheats (average of 4 varieties)											
Yield (g/plot)	60.2	71.0	66.8	66.0	53.7	68.3	66.2	68.6	70.2	7.1	9.4
Germination (per cent)	97.1	96.1	46.4	94.4	49.8	96.3	96.6	96.8	96.6	10.3	13.7
Stem rust (per cent)	58.9	47.7	37.9	45.8	35.0	51.2	49.6	48.7	45.0	8.6	11.4
Kernel weight (g/100)	1.60	1.69	1.74	1.70	1.70	1.70	1.76	1.72	1.77	.12	.16
Barleys (average of 2 varieties)											
Yield (g/plot)	133.8	113.7	84.7	98.7	88.6	138.6	140.9	128.4	139.0	25.0	33.0
Germination (per cent)	97.7	97.4	88.4	97.7	84.0	98.7	98.4	98.4	98.7	12.5	16.5
Stem rust (per cent)[a]	73.1	46.2	47.5	42.5	45.0	56.2	57.5	62.5	52.5	10.9	15.6
Kernel weight (g/100)	2.56	2.2	2.1	2.1	2.0	2.60	2.69	2.61	2.54	.16	.21
Straw plus grain (g)	365.4	353.7	229.4	311.9	309.5	364.7	374.7	360.5	363.0	51.7	68.5
Oats (average of 2 varieties)											
Yield (g/plot)	129.2	139.7	123.1	138.5	121.6	141.4	142.7	151.1	136.9	25.0	33.0
Germination (per cent)	93.5	88.0	48.1	82.0	27.2	82.6	91.0	88.1	87.9	12.5	16.5
Stem rust (per cent)	42.8	26.9	18.1	26.3	13.8	35.6	34.4	33.1	33.1	9.1	12.1
Kernel weight (g/100)	2.69	2.68	2.66	2.69	2.53	2.81	2.68	2.69	2.73	.16	.21
Straw plus grain (g)	392.5	424.2	372.1	419.6	378.6	412.6	415.6	450.5	413.2	51.7	68.5

[a] Recorded only for the variety Custer.

TABLE 2.—*Various characteristics of Mida spring wheat given a single treatment with calcium sulfamate or sodium sulfanilate at different stages of growth*

Stage of growth	Treatment				
	Calcium sulfamate[a]			Sodium sulfanilate[b]	
	Yield	Germination	Wt. of 100 kernels	Yield	Wt. of 100 kernels
	g/plot	per cent	g	g/plot	g
Untreated[c]	36.6	99.7	1.617	35.5	1.564
Tillering	21.6	97.3	1.477	32.0	1.396
1 week after tillering	37.3	99.3	1.567	47.0	1.615
Boot	37.0	97.7	1.715	40.0	1.730
Bloom	46.6	98.0	1.862	40.0	1.565
6 days after bloom	44.3	48.3	1.910	58.5	2.081
11 days after bloom	41.0	77.0	1.707	37.0	1.708
LSD—0.05 for treatment means	9.3	11.4	0.190	7.2	0.274
LSD—0.01 for treatment means	12.9	15.9	0.260	10.7	0.404
LSD—0.05 for treatment vs check	11.1	14.0	0.233	8.8	0.333
LSD—0.01 for treatment vs check	15.5	19.4	0.323	13.1	0.493

[a] Average of 3 replications.
[b] Average of 2 replications.
[c] Average of 6 replications with calcium sulfamate and 4 with sodium sulfanilate.

the untreated plots occurred with treatments made during bloom and 6 days after bloom.

Sodium sulfanilate significantly increased yield when applied either 1 week after tillering or 6 days after blooming and caused a significant increase in weight of kernels when applied at the latter stage. At no stage did it reduce the yield of the grain.

DISCUSSION.—Calcium sulfamate meets most of the requirements of a chemotherapeutant for the control of stem rust of wheat. It is soluble in water in effective concentrations, its action is systemic within the plant, and under certain conditions it will stop the development of stem rust. Two applications at 15 lb. per acre have been observed to stop completely the development of both leaf rust and stem rust in the field (4). In the present investigations, 2 applications of either 9 or 12 lb. per acre gave moderate control (Table 1), and a single application was nearly as effective. Two applications of 12 lb. per acre, however, were ineffective in Mexico during the summer of 1953 when environmental conditions were very favorable for the development of disease.

In the present work, calcium sulfamate increased yield in several cases. The major detrimental effect was a reduction on germination, especially at the heavier doses.

Sodium sulfanilate has caused no detectable detrimental effects on grains in the research conducted to date. It gives good control of stem rust in greenhouse screening tests when applied as a spray to the run-off stage but does not give adequate control in the field when applied at low volumes. This suggests that it has only limited systemic activity.

Several factors must be considered in determining whether either calcium sulfamate or sodium sulfanilate should be applied to grain fields. A single application of calcium sulfamate and 3 of the 4 applications of sodium sulfanilate significantly increased the yield of the spring wheat varieties in 1 test (Table 1). These increases were associated with small decreases in the severity of stem rust. It is possible that the increases in yield are a result of the reduction in rust. It is also possible that the effect on the rust organism was greater than was evident macroscopically. This indicates that complete control of stem rust by a chemical may not be necessary.

The stage of growth at which calcium sulfamate is applied has a definite influence on the degree of injury obtained. A significant reduction in yield was obtained with an application during the formation of tillers, and a significant reduction in germination with an application following blooming. These 2 critical periods are similar to those associated with injury to grains following applications of 2,4-D where it has been found that the 2 critical periods in the growth of wheat and barley are 1) seedling stage when the plants are 1-5 in. tall and 2) the period from early boot to a few days before heading (2, 5, 7).

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THE CHEMICAL COMPOSITION OF LEAVES OF DISEASED TOBACCO: RING SPOT, BACTERIAL WILT, AND FUSARIUM WILT [1]

Frederick A. Wolf and Frederick T. Wolf

SUMMARY

This report contains the results of analyses for total nitrogen, protein nitrogen, total sugars, and petroleum ether extract of comparable samples of diseased and healthy leaves of flue-cured tobacco.

Ring spot results in a significant increase in the nitrogen fraction and a significant decrease in total sugars. Leaves from plants with bacterial wilt contain significantly greater amounts of total nitrogen and of petroleum ether extract and significantly smaller amounts of sugars than do those from healthy plants. Fusarium wilt results in a significant decrease in sugars and a significant increase in petroleum ether extract.

The effects of these 3 diseases upon the relative amounts of these constituents are consistent with the deleterious effects of the diseases on the quality of the crop.

The damage resulting from diseases of crop plants is usually recorded in terms of percentage of plants affected, reduction in yield, or decrease in monetary value of the crop. Although it is generally appreciated that attack of a plant by a pathogen is accompanied by physiological and morphological changes, relatively little attention has been given to the physiology of disease or to the making of analyses to measure the extent of interactions between pathogen and suscept. Studies of the anatomy of diseased tissues have contributed to a limited degree only to an understanding of these interactions. Measurements of alterations in the rates of respiration (1, 7, 13, 15, 17), transpiration (3, 12), and photosynthesis (5, 9, 10, 13) have been made in several kinds of diseased plants. Sempio (13) reported the results of studies of functional imbalances among photosynthesis (P), glycolysis (G), and respiration (R) that occur when wheat is attacked by *Erysiphe graminis* DC. Assuming 1:1:1 as the P:G:R ratio characteristic of healthy wheat, he found that this ratio is changed to 1.71:1.23:1.19 in the initial stages of infection and that it becomes 0.45:0.56:3.82 in the final stages of the disease.

Disturbances in metabolic activities that occur in diseased plants have been shown in a few instances by the results of comparative chemical analyses of diseased and healthy plants. Thus Bunzel (4) found a reduction in the sugar content of beets affected by curly top. Dunlap (7, 8) reported a decrease in total nitrogen and an increase in carbohydrates in tissues of peach, aster, ragweed, and plum infected by yellows viruses. In tissues of tobacco, tomato, squash, cucumber, pepper, and raspberry infected by mosaic viruses, however, both total nitrogen and carbohydrates were increased. Rosa (11) found that a progressive accumulation of carbohydrates occurred in a virus-induced yellow blight of tomato, and True and Hawkins (14) noted marked increases in both sucrose and starch in leaves of virus-blighted spinach. Wolf and Wolf (16) found ratios of total nitrogen to carbohydrates of 1:3 to 1:6 in tobacco affected by the black shank disease as compared with a ratio of approximately 1:8 in leaves of a healthy crop.

It was the purpose of the present work, by means of comparative analyses of diseased and normal leaves of flue-cured tobacco, to determine the extent to which certain constituents are altered in ring spot, caused by tobacco ring spot virus; bacterial wilt, caused by *Pseudomonas solanacearum* E.F.Sm.; and Fusarium wilt, caused by *Fusarium oxysporum* var. *nicotianae* Johnson. Such findings should contribute not only to the theoretical aspects of suscept-pathogen interactions but should also be of practical value to growers and manufacturers of tobacco.

MATERIALS AND METHODS.—Several collections of leaves from diseased plants and from healthy ones of the same variety were made throughout the harvesting season. All infections were naturally occurring ones. Thus, in the case of Dixie Bright 101 tobacco affected by ringspot and the variety Gold Dollar with bacterial wilt, the sample numbers indicate successive dates of

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sampling. In the case of the Hicks variety affected by Fusarium wilt, however, all of the collections were made on the same day. Each collection consisted of paired samples, and each sample was composed of 12–20 leaves, each leaf usually being taken from a different plant. Since the chemical composition of the tobacco leaf is known to be determined in part by its position on the stalk (6), each paired set of samples was taken from corresponding stalk positions.

Immediately after collection, the leaves were fastened to sticks, were allowed to yellow, and were "flue cured" in the manner customarily followed by growers of this type of tobacco. After completion of curing, the leaves were stored in the laboratory for about 4 months. They were then dried and milled to a powder that would pass through a 1-mm screen. Portions of each sample were analyzed for total nitrogen, protein nitrogen, total sugars, and petroleum ether extract (a fraction containing resins, waxes, and related substances) according to the *Official Methods of Analysis* of the Association of Official Agricultural Chemists (2). All results are expressed as percentages on a dry-weight basis. The Fisher "t-test" for paired comparisons was used to determine the significance of the differences in the various constituents found to exist between the healthy and diseased leaf samples.

RESULTS.—The total nitrogen content of 9 samples of ring-spot-affected leaves averaged 39 per cent higher than that of healthy leaves (Table 1). Correlated with the increased total nitrogen of diseased leaves was an increase of the same magnitude in protein nitrogen and a decrease in the total sugars from 28.49 per cent to 17.14 per cent. The 2 kinds of leaves did not differ significantly with respect to the petroleum ether extract.

Somewhat similar results were obtained from analysis of a series of 6 samples of Gold Dollar tobacco affected by bacterial wilt and of corresponding healthy leaves (Table 2). The total nitrogen in the diseased leaves was 24 per cent greater than in healthy leaves,

TABLE 1. *Comparative analyses[a] of ring-spot-affected and healthy leaves of tobacco, variety Dixie Bright 101*

Lot[b]	Total nitrogen		Protein nitrogen		Total sugars		Petroleum ether extract	
	D	H	D	H	D	H	D	H
1	3.76	2.13	1.34	0.77	8.39	32.20		3.22
2	2.39	1.73	1.04	0.87	21.15	30.98	2.85	4.74
3	1.95	1.35	0.81	0.52	21.12	30.37		
4	2.70	2.62	1.21	1.31		18.81		3.52
5	2.00	1.30	1.01	0.64	23.31	32.56		4.54
6	2.44	1.57	1.03	0.66	14.00	34.56	4.00	3.59
7	2.72	1.90	1.07	0.75	14.54	28.80	5.10	4.12
8	2.56	2.11	0.94	0.73	12.74	22.38	6.30	5.04
9	1.95	1.50	0.87	0.63	21.87	25.71	3.29	4.51
Average[c]	2.49†	1.80	1.04†	0.76	17.14‡	28.49	4.31	4.16

[a] Analyses are reported as percentage of the dry weight.
[b] Each lot consisted of paired samples, 1 of diseased leaves (D) and 1 of healthy leaves (H).
[c] Significance at the 1 per cent level is indicated by †; at the 0.1 per cent level by ‡.

TABLE 2.—*Comparative analyses[a] of bacterial-wilt-affected and healthy leaves of tobacco, variety Gold Dollar*

Lot[b]	Total nitrogen		Protein nitrogen		Total sugars		Petroleum ether extract	
	D	H	D	H	D	H	D	H
1	2.39	1.76	0.74	0.70	7.09	25.32	8.26	
2	2.89	1.79	1.03	0.58	6.07	22.69	5.32	
3	1.60	1.50	0.68	0.58	11.31	25.87	4.97	4.27
4	1.53	1.40	0.65	0.56	7.91	20.36	6.80	5.41
5	2.28	2.01	0.64	0.77	7.58	23.95	6.07	4.53
6	1.62	1.43	0.63	0.62	10.12	13.66	7.67	6.86
Average[c]	2.05†	1.65	0.73	0.63	8.35‡	21.97	6.51‡	5.27

[a] Analyses are reported as percentage of the dry weight.
[b] Each lot consisted of paired samples, 1 of diseased leaves (D) and 1 of healthy leaves (H).
[c] Significance at 5 per cent level is indicated by †; at 1 per cent level by ‡.

a significant difference. Differences found in the protein nitrogen fraction were not significant, however. The total sugars, which composed 21.97 per cent of the healthy leaves, were reduced to 8.35 per cent in diseased leaves. The petroleum ether extract also was significantly higher in the diseased leaves than in the healthy ones.

In the case of Hicks tobacco affected by Fusarium wilt, data based on analyses of 7 samples indicate no significant increases in either total nitrogen or protein nitrogen in diseased leaves (Table 3). There was a very marked reduction in total sugars, from 20.78 per cent in healthy leaves to 6.49 per cent in diseased leaves. The petroleum ether extract was significantly greater in diseased than in healthy leaves.

The disequilibria that result in diseased plants perhaps may be most easily seen from comparison of the values for total nitrogen and total sugars. This ratio was approximately 1:7 in ring-spot-affected leaves and 1:14 in the corresponding healthy ones; 1:4 in leaves affected by bacterial wilt and 1:13 in the healthy leaves; and 1:3 and 1:10 in Fusarium-wilt-affected and healthy leaves, respectively.

DISCUSSION.—It was apparent to one with experi-

TABLE 3.—*Comparative analyses[a] of Fusarium-wilt-affected and healthy leaves of tobacco, variety Hicks*

Lot[b]	Total nitrogen		Protein nitrogen		Total sugars		Petroleum ether extract	
	D	H	D	H	D	H	D	H
1	2.69	2.28	1.34	1.14	3.85	17.86	4.54	4.03
2	2.56	2.37	1.21	1.08	4.47	14.38	4.52	3.97
3	2.17	2.33	0.91	0.82	6.35	19.38	7.17	6.23
4	2.21	2.00	0.88	0.72	11.10	24.39	6.14	5.15
5	2.49	1.96	0.85	0.87	4.27	21.12	6.89	5.16
6	2.50	2.11	1.14	0.80	6.50	21.97	5.94	5.15
7	2.24	1.65	1.00	0.61	8.90	26.33	6.79	5.33
Average[c]	2.41	2.10	1.05	0.86	6.49‡	20.78	5.99†	5.00

[a] Analyses are reported as percentage of the dry weight.
[b] Each lot consisted of paired samples, 1 of diseased leaves (D) and 1 of healthy leaves (H).
[c] Significance at 1 per cent level is indicated by †; at 0.1 per cent level by ‡.

ence in appraising quality, by use of the senses of sight, touch, and smell, that all the samples of cured leaves from diseased plants were of less desirable quality than those from comparable healthy plants. This fact becomes meaningful when it is borne in mind that subjective judgment of quality of flue-cured tobacco has been shown to be supported by findings from chemical analyses. For example, good quality is correlated (6) with a wide carbohydrate-nitrogen ratio, i.e. the higher the carbohydrate content and the smaller the content of nitrogenous constituents, the better the quality. The results in Tables 1-3 clearly show that this ratio is greatly and adversely affected in the case of each disease.

Chemical analyses of flue-cured tobaccos have also shown that the quantity of petroleum ether extract is generally least in the better grades of leaf and lug (6). No significant change in this constituent was found in ring spot disease, which is characterized by local areas of necrosis, but in both wilt diseases the quantity of petroleum ether extract was found to be significantly larger. A plausible explanation for such increased quantity is that these wilts cause the plants to be stunted and the leaves to be smaller. Consequently the diseased leaves bear a larger number of glandular hairs per unit area than do healthy ones. These glandular hairs secrete materials that are extractable by petroleum ether.

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A HELMINTHOSPORIUM STEM ROT OF CACTI [1]

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SUMMARY

A top or basal rot caused by *Helminthosporium cactivorum* Petr. has been observed in southern California nurseries on several genera of cacti. Initial symptoms are well-defined yellow lesions that later become dark brown. Entire plants may rot in 2–4 days. In older plants the fungus enters through stomata or wounds, while in young plants direct penetration is also common. The mycelium is intercellular and produces conidiophores that break through the epidermis or stomata. Abundant sporulation occurs under moist conditions. Successful control has been achieved by 1) applying 2–4 lb. captan per 100 gal. plus 6 fl. oz. DuPont Spreader-Sticker on a 10–14 day schedule, 2) roguing diseased plants, and 3) sterilizing the soil.

In the spring of 1954 a very destructive stem rot was first identified on seedlings of several genera of cacti in southern California nurseries. The disease was largely restricted to seedlings less than 3 in. high and younger than 2 years. Losses in some species commonly reached 50 per cent. All plants in individual flats often were diseased. The growing of cactus plants is so difficult, largely because of disease losses, that few growers will undertake their production from seed. Of the more than 40 growers of cactus and succulents in southern California, only 6 produce the majority of the estimated 1½ to 2 million seedlings grown here annually.

Seedlings are grown in flats for 2–6 months, depending upon the variety. They are then transplanted into other flats, 400 per flat. At this stage the tender cotyledons are easily injured in handling, but the spines, if present, have not hardened and cause no injuries. The plants grow rapidly and within 6 months to a year are in contact and ready for a second transplanting, 100 per flat. Some varieties, however, are ready for sale at this time. As the spines harden, injuries occur more frequently because of punctures or breakage of spines. It is during this period that the disease causes greatest loss. When the seedlings have become crowded, they are dried off and dug, shaken free of soil, and thrown in piles for 24 hours or longer. They are shipped in this condition in either boxes or bags, or they may be potted for later sale. In either case many injuries result and infection commonly occurs.

Although nurserymen have seen the disease in this area for many years, a literature survey indicated that the only previously published report of its occurrence in the United States was from Texas where it was found on *Echinocereus chloranthus* (Engelm.) Rumpler (8). The disease was first reported in Czechoslovakia by Petrak in 1928-29 on seedlings of 5 genera of cacti (7). He believed that the causal Helminthosporium had been introduced on seed from Mexico in 3 successive years, since the disease did not appear in plants grown from other seed sources. It was observed in Italy prior to 1932 on 3 genera of cacti (2). Flachs (4) observed the disease in Germany on 5 genera in 1933 and considered that the causal agent was introduced with seedlings from Mexico.

SYMPTOMS.—The disease in California apparently is restricted to seedling cacti and is most serious in very young plants, decreasing in occurrence as the plants mature. While this incidence is partly due to greater susceptibility in the seedling than in later stages, cultural operations are a contributing factor.

In young seedlings, infections are likely to develop on the cotyledons, and the plants rot rapidly. In older plants the disease usually appears as a top rot beginning on the shoulder or growing point or, sometimes, as a basal rot occurring near the soil surface. At first the lesions appear yellow, but these rapidly enlarge and become dull dark green and water-soaked, or dark brown, depending on the conditions and species. The areas have well defined margins and may be either unilateral or uniform on all sides. Under continued moist conditions the entire plant is converted within 2–4 days into a watery mass contained by the epidermis. Under dry conditions the infected tissues collapse, the lesion appears as a smooth sunken dark brown area, and enlargement practically ceases. With the return of moist conditions, the fungus rapidly spreads and a dark brown mass of spores is formed. The final stage in any case is a shrunken dark brown mummy covered with spores (Fig. 1).

Certain other diseases of cacti might be confused with this one. *Phytophthora cactorum* (Leb. & Cohn) Schroet. and *Pythium debaryanum* Hesse cause root and collar rots (6) in which the affected tissues also become soft and watery and vary from brown to black. These water molds, however, always attack from the base upwards, the roots are involved, and the dark brown sporulation typical of the Helminthosporium disease is lacking. *Fusarium oxysporum* (Schlecht.) Snyd. & Hans. and *F. episphaeria* (Tode) Snyd. & Hans. cause tip and basal rots (5, 6, 9) but may be distinguished from the Helminthosporium disease on the following bases: the dark brown to black lesions have persistent yellow margins, the rot is dry and

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slow spreading, and the affected tissues may show masses of pink sporulation. *Rhizoctonia solani* Kühn also produces a collar rot in cacti, but in this case the tissues assume a brown, shrivelled, dry appearance. Coarse mycelial strands may be seen with a hand lens, and no sporulation occurs.

HOST RANGE.—The fungus apparently is restricted to the Cactaceae within 5 subtribes of the tribe Cereeae, according to the classification established by Britton and Rose (3). It occurs on 16 spp. or varieties of Mammillaria, 13 of Echinocactus, 9 of Echinocereus, 7 of Cereus, 7 of Cephalocereus, 2 of Lophocereus, 2 of Lemaireocereus, and 1 each of Espostoa, Pilocereus, Selenicereus, Astrophytum, and Ferocactus. Under recent classifications, the hosts of the fungus occur in the subfamily Cereoideae and with but 1 exception (Selenicereus of the tribe Hylocereae) are all in the tribe Cereeae. This tribe is further broken down into 2 sections: 1) those plants whose origin lies south of the tropical belt, and 2) those plants whose origin lies north of the tropical belt (1). It is interesting to note that all of the affected genera fall into the latter group, and that the native habitat for most of them ranges from Mexico to the southwestern United States.

New hosts reported in this list include *Cereus peruvianus* (L.) Miller, *Cephalocereus tetetzo* (Weber) H. Bravo, *C. collinsii* Br. & R., *C. sartorianus* Rose, *C. mezcalaensis* H. Bravo, *Lophocereus schottii* (Engelm.) Br. & R., *L. gatesii* Jones, *Mammillaria hahniana* Werd. & Bckbg., *Espostoa lanata* (HBK.) Br. & R., *Selenicereus* sp., *Lemaireocereus martinezi* Ortega, *L. dumortiei* (Scheidweiler) Br. & R., *Astrophytum ornatum* (DC.) Weber, and *Ferocactus* sp.

THE PATHOGEN.—The organism consistently isolated from diseased plants was *Helminthosporium cactivorum*, which was described and named in 1931 by Petrak (7). In 1932 Bongini (2) studied this disease and, unaware that the fungus had already been described, named the pathogen *Helminthosporium cactacearum*. She was undoubtedly dealing with the organism involved in the present study and the one described by Petrak, whose binomial is the accepted one for this fungus. The fungus studied in California agreed in all particulars with that described by Petrak.

The conidiophores of the fungus are erect, elongate, brown, rarely branched, irregularly nodulose, and lighter colored at the tip which is usually somewhat swollen and denticulate; the conidial scars are clearly visible. The conidiophores measure from 30–280 × 4–13 μ and occur in dense fascicles, rarely singly, breaking through either the epidermis or stomata. The conidia are borne singly; they are hyaline at first, later becoming thick-walled and dark olive to chocolate brown. They are 1- to 5-septate (usually 3) and for the most part are cylindrical and straight with rounded ends, but occasionally bifurcate types may be found (Fig. 2). The conidia are borne acrogenously, but sometimes the new growing point is so close to the previously borne spore that they appear to be in verticillate whorls. The range in spore size reported by Petrak and Bongini from diseased material was 12–75 × 6–13 μ. Spores from the host measured by us were 19–59 × 9–14 μ (ave. 40 × 12 μ). Spores occur copiously on dead plant parts, and field evidence indicates that these are

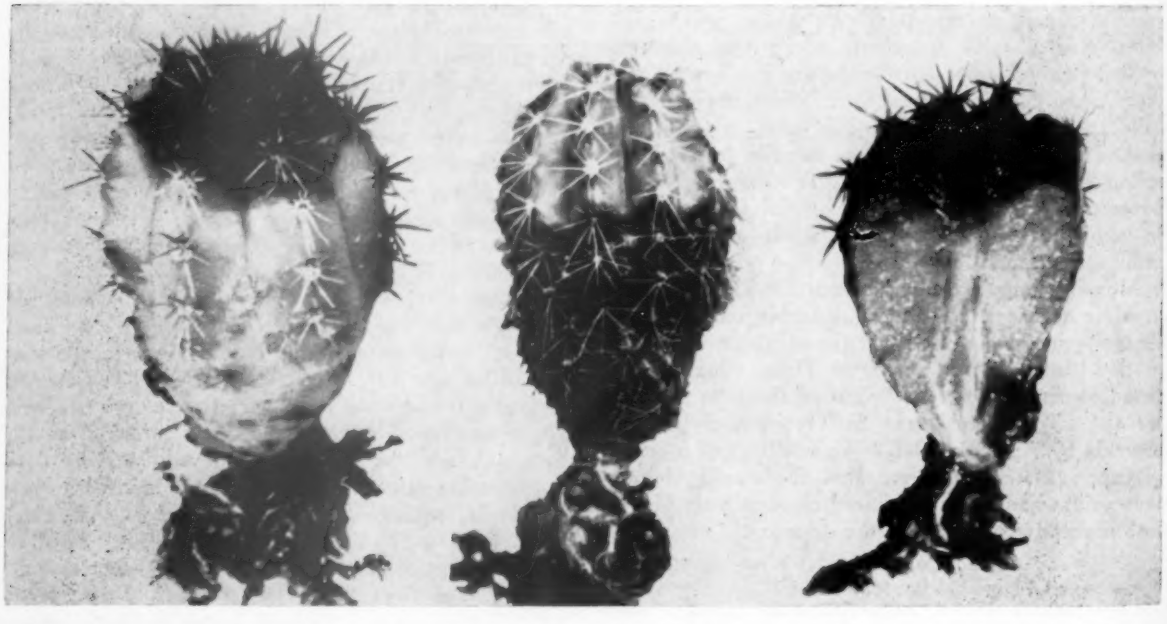

FIG. 1. Left to right: top rot on *Cephalocereus tetetzo*, bottom rot on *C. mezcalaensis*, and internal view of *C. tetetzo* showing cortical rot and vascular discoloration.

spread by air currents, as is common with other Helminthosporia. The perfect stage was found neither in nature nor in culture.

The disease was reproduced by spraying aqueous spore suspensions of the organism on 60 plants, 8-months-old, of *Cephalocereus tetetzo*. Isolations from inoculated plants yielded the original organism. Free-hand and frozen sections of infected tissues revealed much cell disorganization and break-down of chloroplasts. The mycelium is intercellular, hyaline, densely granular, and aggregates in the subepidermal tissues into parallel rows of hyphae that form light brown stromatic masses from which the conidiophores arise (Fig. 2). Because of possible confusion of mycelium of this fungus with that of *R. solani* in host tissue, for identification in the absence of sporulation it is advisible to culture from diseased material.

The conidiophores of *H. cactivorum* are short and densely fasciculate under dry conditions, but become elongate, spreading, and wavy under very moist conditions. In rotten plants the hyphae give rise to spherical elements resembling chlamydospores that are brown, heavy-walled, and filled with oil droplets.

In studies on the mode of entry of the pathogen into the host, water suspensions of conidia from single-spore cultures were sprayed onto disease-free cactus seedlings of various genera held on moist filter paper in Petri dishes. The seedlings ranged in age from 1½ weeks to 4 months. Except for the youngest plants, some were injured by pricking with a needle and the rest left uninjured. All of the tiny seedlings became infected when inoculated. Epidermal strips from the older plants were made at 3-hour intervals and mounted in lactophenol cotton blue to determine the time and method of penetration. It was found that free moisture was essential for penetration, otherwise the germ tubes dried and germination ceased. Appressoria formed readily after 18–21 hours, and after 48 hours penetration had occurred directly through the epidermis. In only a single instance was stomatal penetration observed. Within 3 days sporulation occurred over the entire surface of all injured plants. A few uninjured plants exhibited yellowish brown lesions, and on the fifth day sporulation developed. When 1½-year-old plants of *Cereus peruvianus* were used, direct penetration did not occur, although instances of stomatal penetration were observed. When the plants were injured, penetration occurred readily and the plants rotted within a week.

Bongini (2) found that inoculations were always

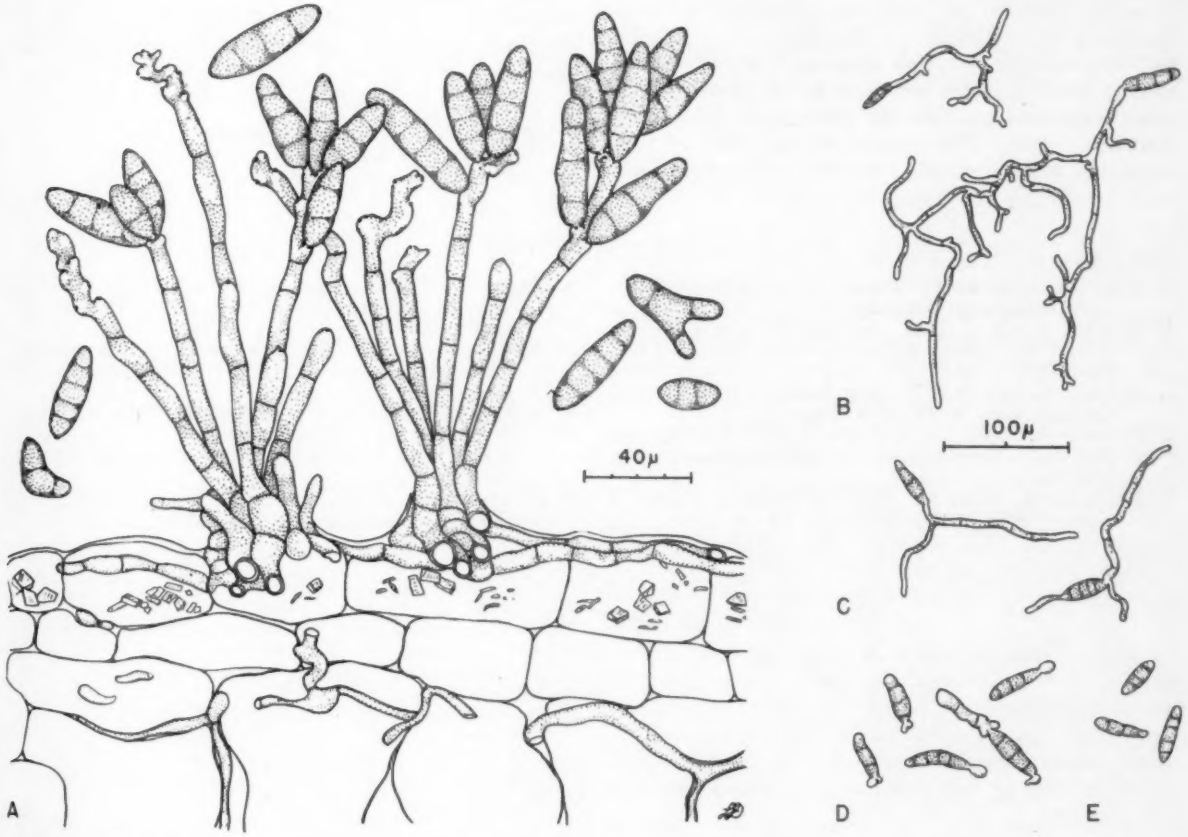

FIG. 2. *Helminthosporium cactivorum*. A) Conidia and conidiophores arising from stromatic masses in host tissue. B-E) Effect of fungicides on spore germination after 4 days. B) Check, C) zineb, D) captan, and E) ferbam.

successful on young pricked plants when the inoculum was placed near the collar, but many times the lesions at the apex healed over with no further disease expression. Inoculations of young uninjured plants with spore suspensions were for the most part ineffective, as were those on plants a year or more old, whether injured or not. Our tests confirmed her results except when 1½-year-old plants were used.

CONTROL.—An exploratory glass-slide assay, using a suspension of Helminthosporium spores, was run to find an effective fungicide. In 13 hours there was no germination on slides sprayed with captan (*N*-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide) or ferbam (ferric dimethyldithiocarbamate) at 2 lb. per 100 gal. Spore germination was approximately 3 per cent on slides sprayed with zineb (zinc ethylenebis[dithiocarbamate]) at 2 lb. per 100 gal. and 100 per cent on control slides sprayed with water. After 4 days there was still no germination on slides sprayed with ferbam, and on those sprayed with captan spores bore abnormal "knobs". Germination was 100 per cent in the presence of zineb and in the controls (Fig. 2).

As a result of these tests, spray trials with ferbam, captan, and zineb were run on seedlings of *Cephalocereus tetetzo* 1½ in. high (slightly more than 1 year old) in flats. Fungicide concentrations equivalent to 2 and 4 lb. per 100 gal., plus 6 fl. oz. DuPont Spreader-Sticker, were employed, and 40 plants were used for each treatment. The sprays were applied and allowed to dry, then the plants were heavily inoculated with a spore suspension and covered with plastic and cheesecloth for 1 week. Effective control was obtained with captan or ferbam at either the 2- or 4-lb. level on a 10- to 14-day schedule. Ferbam is objectionable for use on cactus seedlings, however, because of the conspicuous black spray deposit that collects in the spine cushions. Zineb was ineffective, even at the 4-lb. level. The percentages of diseased plants after 17 days were 52.3 for the inoculated check, 14.5 for the noninoculated check, 14.6 for zineb, 5.5 for ferbam, and 4.0 for captan.

The effect of dry heat on the mycelium in the host tissues was investigated. The fungus was recovered in every case by culturing from infected plants that had been held in a drying oven at 60°C for 22 hours. The plants were not able to withstand this treatment. Therefore, heat therapy does not seem to offer any promise as a means of salvaging damaged plants.

In the nursery a complete sanitation program is necessary for effective control. All diseased plants should be removed from the flats at intervals and burned to reduce the inoculum potential of air-borne spores. A soil sterilization program using either steam or chemicals should be practiced to eliminate possible carry-over of the pathogen. It would be a wise precaution not to plant new seedlings in a glasshouse where old infected plants are growing. Captan sprays (2 lb. per 100 gal., plus 6 fl. oz. DuPont Spreader-Sticker) should be applied on a 10-day schedule. A commercial nursery that followed the procedures resulting from this work has achieved successful control.

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TYPES OF SEEDLING REACTION OF KAWVALE AND WABASH WINTER WHEAT TO THREE PHYSIOLOGIC RACES OF USTILAGO TRITICI [1]

R. E. Ohms and Wayne M. Bever [2]

SUMMARY

Wabash and Kawvale, winter wheat varieties, were inoculated with races 1, 3, and 11 of *Ustilago tritici* (Pers.) Rostr. There was no difference in the percentage of embryos infected in Kawvale and Wabash inoculated with the 3 physiological races. Two weeks after seeding, the third internodes of infected seedlings had reduced elongation with no fourth internode elongation in Wabash in contrast to a reduced elongation of the third internodes and an elongation of the fourth internodes in Kawvale. Three weeks after seeding, the fourth and fifth internodes of both Wabash and Kawvale infected seedlings were abnormally elongated.

Resistance in Kawvale was expressed in the seedlings before the third foliage-leaf stage. In this type of resistance the scutella were infected, but the growing points were not. Resistance appeared to result from the ability of the seedling to outgrow the invading fungus.

The low percentage of smutted heads of Wabash inoculated with race 11 was due to an over-susceptibility in the seedling stage. Seedlings with growing-point infection either failed to emerge or died between the third and fifth foliage-leaf stages. Prior to emergence, Wabash seedlings infected with *U. tritici* were more susceptible to infection by soil organisms.

The breeding for resistance to loose smut, caused by *Ustilago tritici* (Pers.) Rostr., and the determination of physiologic races of the fungus have been based on the adult-plant reaction. Very little, if any, consideration has been given to the existence of identifiable seedling reactions. It has been more or less assumed that the resistance of varieties to *U. tritici* was due to the inability of the fungus to keep pace with the growth of the host. It was not until 1953, when Oort (7) published his results on a "hypersensitive reaction" in the seedling stage, that attention was directed towards a second type of reaction.

A stable character whereby a plant infected by *U. tritici* could be easily identified in the seedling stage would facilitate the identification of physiologic races and breeding for resistance.

The investigation here reported was undertaken to study the nature of resistance to *U. tritici* and to determine morphological responses of infected winter wheat seedlings.

In the following discussion, McCall's (5) internode description was followed in designating the growth stages and seedling morphology.

In 1954, the writers (6) reported that the extent of elongation of the fourth internode could be used for the determination of resistance or susceptibility to a physiologic race of *U. tritici*. No differences were observed in the frequency of infection of the embryos of Kawvale C.I. 8180[3] (resistant) and Wabash C.I. 11384 (susceptible). Wabash seedlings infected with race 3 developed abnormally short third internodes and non-elongated fourth internodes. In Kawvale seedlings infected with race 1, 3, or 11, the third internode was short and the fourth was elongated. Normal elongation of the third internode indicated that a seedling was not infected with *U. tritici*. In infected seedlings, hyphae could be observed in both scutella and growing points of Wabash but only in the scutella of Kawvale.

Popp (8) and Vanderwalle (10), working with spring wheat, and Ohms and Bever (6), working with winter wheat, reported that the embryos of resistant varieties were infected with *U. tritici* as frequently as were those of susceptible varieties. Popp (8), Vanderwalle (10), and Larose and Vanderwalle (4), however, found that the embryos of some resistant varieties of spring wheat were not infected. Thus, there appears to be a type of resistance characterized by the inability of *U. tritici* to infect the embryos of spring wheat. Caldwell and Compton (3) suggested that susceptible winter wheat embryos, of hybrid origin, were not infected because a resistant pericarp enclosed a susceptible embryo.

A type of resistance in which the scutella were infected but the seedling growing points were free of infection was reported by Popp (8) in spring wheat and by Ohms and Bever (6) in winter wheat. Popp found that seedlings of 1 resistant variety of spring wheat showed growing-point infection but no smutted heads.

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Approved by the Director of the Illinois Agricultural Experiment Station. Cooperative investigations of the Field Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture, and the Illinois Agricultural Experiment Station. Portion of thesis submitted in partial fulfillment of the requirements for the Ph.D. degree at the University of Illinois.

[2] Formerly Assistant in Agronomy (Crop Pathology), now Assistant Pathologist, South Dakota Agricultural Experiment Station; and Agent (Pathologist), Field Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture, and Professor of Plant Pathology, Department of Agronomy, University of Illinois, respectively.

[3] C.I. refers to the accession number of the Cereal Crops Section.

A hypersensitive reaction in wheat to *U. tritici* was reported by Oort (7). The infected seedlings died in the second to fifth foliage-leaf stages. The infected seedlings were shortened and curled and showed chlorotic longitudinal striping.

MATERIALS AND METHODS.—Flowers of 2 varieties of winter wheat, Wabash and Kawvale, were inoculated by the hypodermic needle method with races 1, 3, and 11 (1). According to Bever (2), Wabash is resistant to race 11 but is susceptible to races 1 and 3, and Kawvale is resistant to all 3 races. The embryos were prepared for examination by the sodium hydroxide method of Simmonds (9). The clearing and staining procedure followed that of Ohms and Bever (6). The embryos were washed several times with boiling water and then placed in a nearly saturated aqueous solution of chloral hydrate (4.5 parts chloral hydrate to 1 part water) containing 0.01 per cent acid fuchsin. This solution containing the embryos was heated in an Arnold steamer about 5 minutes or until the embryos were clear. After clearing and staining, the embryos were placed in lactophenol for examination.

To determine the internode elongation as affected by *U. tritici*, the inoculated seeds were sown 3 in. deep in unsterilized soil in 2-gal. glazed jars in the greenhouse. The plants were grown in temperature-control cages at 68–71°F. Plant samples were taken after 14 and 21 days. Seedlings on the fourteenth day were beginning the third foliage-leaf stage and the fifth on the twenty-first day. The seedlings were grouped according to the length of the third internode. The maximum third internode length of infected seedlings was 2.4 in. After classification for internode length, the seedlings were cleared and stained by heating for about 15 minutes in the solution of chloral hydrate plus acid fuchsin. Then the scutella and growing points were smeared in lactophenol and examined for the presence of infection hyphae.

RESULTS.—*Internode elongation reaction.*—Figure 1 shows 14-day-old Wabash seedlings with varying de-

FIG. 1. Seedlings of Wabash from infected seed planted at a 3-in. depth showing A) 80 to 100 per cent, B) 60 to 80 per cent, C) 40 to 60 per cent, and D) 20 to 40 per cent reduced elongation of the third internode. E) Not infected. ×2.4 (Pointers at the fourth node).

FIG. 2. Seedlings of Kawvale from seed planted at a 3-in. depth showing effect of infection by *U. tritici* on internode elongation. A) Not infected. Normal internode elongation. B) Infected. Reduced elongation of third internode with the fourth elongated. ×2.4 (Pointers at the fourth and fifth nodes).

grees of third internode elongation. The 4 plants on the left show reduced elongation of the third internode. These plants contained hyphae in both the scutella and growing points, and there was no apparent elongation of the fourth internode. The plant on right contained no hyphae in the scutellum and the third internode elongated normally. In contrast, Figure 2 shows the type of internode elongation in 14-day-old Kawvale seedlings. The seedling showing a reduction in the elongation of the third internode had the fourth internode abnormally elongated and contained hyphae in the scutellum, but not in the fourth node or growing point. Resistant Kawvale seedlings could be separated from the susceptible Wabash seedling reaction (Fig. 1) on the basis of the fourth internode elongation.

The internode shortening criteria was not absolute as 10.6, 4.4, and 0.0 per cent of the 14-day-old Wabash seedlings contained hyphae in the scutella and growing points and showed no apparent reduction in the length of the third internode when inoculated with races 1, 3, and 11, respectively. The seedlings with reduced internode elongation did contain infection hyphae. As in Wabash seedlings, some of the 14-day-old Kawvale seedlings contained infected scutella, but had no internode abnormalities. The percentage of seedlings with normal internode elongation and infected scutella were 16.7 and 26.4 for races 3 and 11, respectively.

Twenty-one days after planting, the fourth and fifth internodes had elongated in some of the Wabash and in all of the Kawvale seedlings. The types of internode elongation of infected seedlings included 1) third internode short with the fourth and fifth not elongated; 2) the third short, the fourth elongated, and the fifth not elongated; 3) the third short, the fourth not elongated, and the fifth elongated; and 4) the third short with the fourth and fifth elongated.

The 21-day-old Wabash seedlings had scutella and growing point infection, whereas, with the exception of 2 plants, all the Kawvale seedlings contained only scutellar infection. These results were similar to those secured with 14-day-old seedlings. The percentage of infected seedlings that showed no internode elongation in Wabash were 4.4, 0.0, and 0.0, and in Kawvale 6.0, 9.5, and 16.7, respectively, for races 1, 3, and 11.

Embryo, scutellum, and growing point infection.—

TABLE 1.—*Embryo, scutellar, and growing-point infection in Wabash and Kawvale winter wheat by races 1, 3, and 11 of U. tritici*

Variety	Race	Percentage infected			Field reaction
		Embryo	Scutellum	Growing point	
Wabash	1	54.3	48.0	48.0	Susceptible
	3	35.4	31.0	31.0	Susceptible
	11	76.9	5.0	5.0	Resistant
Kawvale	1	48.9	40.8	0	Resistant
	3	59.2	52.4	2.4	Resistant
	11	74.5	78.6	2.4	Resistant

TABLE 2.—*Stand counts on 3-week-old-seedlings of Wabash and Kawvale winter wheat inoculated with races 1, 3, and 11 of U. tritici*

Variety	Race	Number seeds planted	Stand count	Percentage reduction in stand
Wabash	1	50	46	8
	3	50	39	22
	11	50	23	54
Kawvale	1	50	49	4
	3	50	42	16
	11	50	42	16

The percentages of the embryos (from seed examination), scutella (from seedling examination), and growing points infected are given in Table 1. Embryo infection was approximately the same in Kawvale and Wabash. When the scutella in Wabash were infected, the growing points were also. In Kawvale, the growing points were not invaded by race 1, and only 2.4 per cent of the growing points were infected by race 3 and the same percentage by race 11.

The percentage of the plants showing scutellar infection was nearly equal to the percentage of the embryos showing infection. A Chi square analysis showed good agreement between the scutellar (plant) infection and that expected on the basis of the percentage of embryo infection, except in Wabash infected with race 11. The P values are as follows: Wabash—race 1, >0.3; race 3, >0.5; and race 11, <0.01, respectively; and for Kawvale—race 1, >0.2; race 3, >0.3; and race 11, >0.5.

Data on stand count given in Table 2 clarify the reaction of the seedlings. The stand was considerably less than the number of seeds planted in the case of Wabash and Kawvale infected with races 3 or 11. This reduction in stand was not associated with seedling infection except in Wabash infected with race 11. Here the reduction in stand was caused by the death or nonemergence of the infected seedlings as indicated by the P value.

Over-susceptibility reaction.—The facts that the seedlings of Wabash infected with race 11 contained both scutellar and growing-point infection and that there was a decrease in stand indicate that Wabash's field resistance to race 11 is due to an over-susceptibility.

A repeated experiment included 4 treatments of 3 replicates with 38 seeds each. The seed was planted 3 in. deep in soil in 2-gallon glazed jars in a greenhouse with a temperature range of 76–85°F. Wabash seed infected with race 11 and noninfected seed was planted in both steamed and nonsteamed soil. The soil was heated in an autoclave for 20 minutes at 15 lb. pressure.

Emergence was complete 14 days after planting. Stand counts taken at this time were analyzed by the analysis of variance. Significant differences in emergence resulted from both infected seed and soil treatments, but no significant differences due to soil treatment were found when noninfected seed was planted

TABLE 3.—*Stand counts*[a] *of Wabash, 14 and 21 days after the planting of infected and noninfected seed in the greenhouse*

Soil treatment	Seed infected by race 11 of U. tritici		Seed not infected	
	14 days	21 days	14 days	21 days
Steamed	31.3	19.0	35.3	33.3
Nonsteamed	21.7	10.0	34.3	29.7
LSD (0.05)	3.45	5.04	3.46	5.04

[a] Figures are the averages of 3 replicates, each planted with 38 seeds.

(Table 3). It appears that soil organisms may have contributed to the decrease in the emergence of infected seedlings.

There was no noticeable loss of seedlings until after the second week. After 21 days, when the seedlings were between the third and fifth foliage-leaf stages, there was a significant difference among treatments and the responses were in the same direction as at the 14-day stage. There was no noticeable chlorotic striping on the leaves of the dying seedlings, as described by Oort (7), but some of the second, third, and fourth foliage leaves were curled. The surviving seedlings were examined microscopically for the presence of mycelia. Where hyphae were found in the scutellum, infection hyphae were found also in the growing point of the seedling. Chi square analysis was used to test the observed number of infected and noninfected seedlings against the expected numbers calculated from the percentage of embryos infected, 76.9. To determine whether the missing seedlings were from infected seed, the number of infected seedlings was calculated by adding the number of surviving infected seedlings, 27, to the number missing, 141. The number of observed infected seedlings, 168, agreed well with the expected, 175, with a probability of 0.20 to 0.30. This supports the conclusion that the missing plants were from infected seed.

DISCUSSION.—The resistances of Kawvale and Wabash to *U. tritici* were found to differ in type. In Kawvale the growth rates of the infection hyphae probably were too slow to keep pace with the growing point of the host. This type of resistance was previously reported to occur in Kawvale by Ohms and Bever (6) and in spring wheat by Popp (8). The resistant internode reaction of Kawvale could still be distinguished from the susceptible reaction of Wabash when the seedlings were beginning the third foliage-leaf stage but not when the seedlings were beginning the fifth foliage-leaf stage. The percentage of infected seedlings escaping detection by the internode reaction, however, was less at the fifth foliage-leaf stage.

A second type of reaction expressed by Wabash was the death of infected seedlings between the third and fifth foliage-leaf stages. The over-susceptibility to infection by *U. tritici* results in a lower percentage of smutted mature plants; an apparent false resistance. This behavior, and the lack of chlorotic striping on the leaves, distinguishes the reaction from that described by Oort (7).

The resistant and susceptible reactions can be distinguished by macroscopic examination. The Kawvale "resistant reaction" involving the fourth or fifth internode elongating in the second foliage-leaf stage was easily distinguishable from the susceptible Wabash reaction whereby the fourth or fifth internodes did not elongate. The over-susceptible reaction of Wabash was readily distinguished from the susceptible reaction, when the seedlings were in the fifth foliage-leaf stage, because many of the infected plants had died.

The indicated importance of these reactions lies in the rapidity of determining the reaction of a variety to a collection of loose smut, in physiologic race identification, in breeding for the transfer of resistance, in studies of the inheritance of disease resistance, and in the studies of the nature of physiological specificity of the causal agent. Past studies on the inheritance of resistance to *U. tritici* probably are somewhat in error because they were based on the percentage of smutted heads produced in the field.

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A Device for Rapid Inoculation of Citrus Fruits. C. N. ROISTACHER AND L. J. KLOTZ. During investigations on control of decay of oranges and lemons caused by blue-green molds, *Penicillium italicum* Wehmer and *P. digitatum* Sacc., the need for a rapid method of inoculation became apparent. Except for spread by contact from fruit to fruit, infection of citrus fruits by conidia of these *Pencillium* spp. takes place only through wounds. Formerly the technique of inoculation used consisted of scratching a wound 1 cm long by 1 mm deep on the shoulder of the fruit and either dipping the entire fruit into a spore suspension or brushing dry spores into the wound. When it became necessary to inoculate large numbers of fruit within a reasonable period of time the above technique was found too slow and tedious, and it was soon evident that a more rapid method was highly desirable.

The device shown in Figure 1 consists of a Plexiglas container holding a suspension of inoculum in water and also enclosing a circular saw blade which dips into the inoculum and projects 1 mm through a slit in the top. The saw, which rotates at a reduced speed of 210 rpm, carries a film of spore suspension with it while agitating the liquid inoculum, keeping the spores in suspension. Fruit pressed gently on the protruding blade is simultaneously injured and inoculated uniformly.

To make the Plexiglas case, sheets of the material of ⅛-in. thickness are easily cut on a band saw and ground smooth to make the circular outer frame. The narrow piece to make the width of the container is heated at 300°F until it softens and is gently shaped around the 2 outer frame pieces and held in position while the Plexiglas hardens rapidly in place. The pieces of Plexiglas are glued securely together by injecting or brushing ethylene dichloride into the seams. If ethylene dichloride is not available, an acetone-base cement may be substituted. The top piece consists of ¼-in Plexiglas and is held firmly by 4 screws as shown. If so desired it can be made adjustable by cutting vertical slots in the edges of the case.

A 1/30th H.P. sewing-machine motor or any small motor mounted as shown can be used. A sewing-machine pulley, ¾-in. diameter, is mounted at the end of the motor shaft and is connected by ¼-in. round leather belting to a 6-in. pulley. This effects a reduction from 1730 rpm to a saw speed of 210 rpm, or slightly greater than 8 to 1. This speed has been

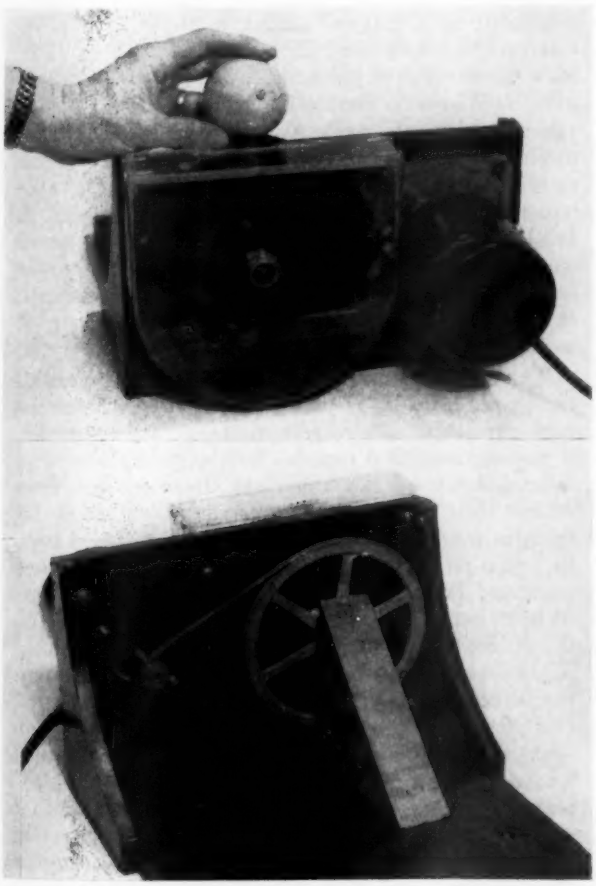

FIG. 1. Front and rear views of inoculating device. The rotating saw dips into the inoculum, carries a film of spore suspension on its teeth, and thus wounds and inoculates at the same time. Note the projecting teeth above Plexiglas top.

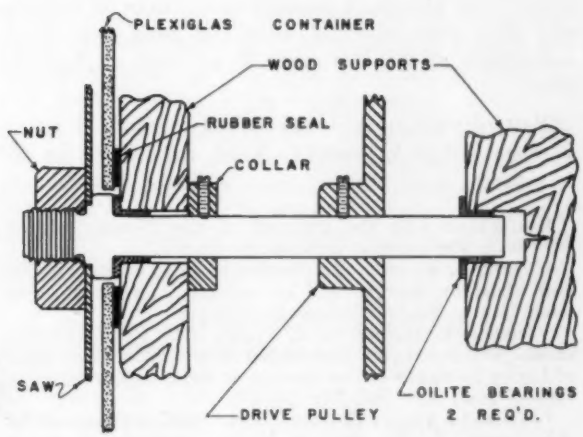

FIG. 2. Special shaft holding saw blade. Shaft rotates between 2 Oilite bearings press-fitted into wood supports. Both the collar and drive pulley are firmly attached to the shaft by set screws.

found satisfactory for our purpose.

A used saw blade can be procured from any saw-sharpening establishment and its edges ground down to approximately 1 mm at the tip. The saw is placed on a special shaft and bolted in place (Fig. 2). After use the blade is easily removed, the inoculum poured out, and the inside of the container rinsed and dried.

In the many tests in which this device was used in the inoculation of oranges and lemons, an average of 98.4 per cent of a large number of fruit developed decay. The portable nature of this inoculator permits taking it to the packing houses for large-scale inoculations in studies on the effects of mold-inhibiting chemicals on spores in wounds. Application of the method in the study of other fruit and bulb decay problems is apparent.—Department of Plant Pathology, Citrus Experiment Station, University of California, Riverside, California.

The Effect of the Water-soak Seed Treatment on the Germination of Certain Barley Varieties Grown at Different Locations.[1] D. C. Arny and Curt Leben. The evidence now at hand[2,3,4] suggests that Tyner's water-soak method and a modification[5] are useful for the control of loose smut in barley. Injurious effects of the treatment on stand, however, have been noted in some varieties[2,3,4]. It was of interest, therefore, to note the influence of the soak treatment on the germination of samples of seed of several barley varieties and to examine the response of seed of given varieties grown at different locations. In the course of this work, further evidence on the effectiveness of the water-soak method for loose smut control also was obtained.

Samples of seed of Kindred, Manchuria, Montcalm, Moore, and Wisconsin Barbless grown in 1953 at 16 locations in the Upper Mississippi Valley Spring Barley Nurseries were supplied by the U. S. Department of Agriculture Barley and Malt Laboratory. The variety Mars was obtained from only 8 of these locations. The remaining samples were from Wisconsin and they were obtained from the Seed and Weed Laboratory of the Wisconsin Department of Agriculture.

Part of each sample was soaked in distilled water in open 125-ml Erlenmeyer flasks at 22–25°C for 56 hours, and the remaining part was not treated. Soaked seed was drained, dried, and stored in envelopes until planting. Germination of treated and nontreated seeds of each sample was determined in soil in the greenhouse. Field tests at Madison in 1954 were used for the determination of the incidence of loose smut.

Table 1.—*Germination of nontreated seeds and of soaked seeds of several barley varieties*

Variety	No. of seed samples	Average germination for all samples	
		Nontreated	Soaked
		per cent	per cent
Kindred	27	96 ± 4[a]	56 ± 14[a]
Manchuria	16	96 ± 4	81 ± 14
Mars	10	94 ± 8	72 ± 22
Montcalm	25	94 ± 6	77 ± 15
Moore	26	90 ± 8	73 ± 18
Oderbrucker	5	94 ± 7	77 ± 6
Wisconsin Barbless	19	93 ± 4	88 ± 7
Total or average	128	93.7	74.9

[a] Standard deviation of single determination.

A summary of the germination data for each variety is given in Table 1. Since the Wisconsin samples responded in a manner similar to those from other locations, the data were all considered together. The soak treatment was particularly harmful to Kindred, whereas Wisconsin Barbless was little affected. Other varieties were intermediate. It is believed that these responses were due to inherent characteristics of the varieties; the effect of location would not seem to be important in view of the number of locations sampled. In the 96 samples of seed that carried loose smut, only 15 produced a few smutted heads following the soak treatment. These 15 were fairly evenly distributed among the varieties, although there were somewhat more in Moore than in the others.

When the data for the 5 varieties obtained from all the Upper Mississippi Valley Spring Barley Nurseries were compared with respect to origin of the samples, it was apparent that samples from certain places were injured more by the treatment than samples from others. For example, the average germination for samples from St. Paul, Minnesota, was reduced from 91 per cent for nontreated to 49 per cent for soaked seed, and for samples from Waseca, Minnesota, from 93 to 62 per cent. In samples from Saginaw, Michigan, the reduction in germination due to soaking was only slight—from 96 to 90 per cent. For other locations, the injury was intermediate. Different environmental conditions at the several locations probably were responsible for the varying degrees of injury produced by the soak treatment. These environmental conditions will vary from year to year. The average amount of loose smut per row in nontreated barleys varied from none in those from DeKalb, Illinois, to 58 smutted heads per 15-ft. row in those from St. Paul.

Ordinarily, when seed germination is reduced moderately, there will be at least partial compensation because of increased tillering of surviving plants. In

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

[2] Tyner, L. E. 1953. The control of loose smut of barley and wheat by Spergon and by soaking in water at room temperature. Phytopathology 43: 313-316.

[3] Russell, R. C., and L. E. Tyner. 1954. The influence of temperature on the time required to control loose smut of barley by means of the Spergon or water-soak treatments. Canad. Jour. Agr. Sci. 34: 533-538.

[4] Leben, C., and D. C. Arny. 1954. Soaking treatment for the control of loose smut of barley. Phytopathology 44: 329-330.

[5] Hebert, T. T. 1955. A new method of controlling loose smut of barley. U. S. Dept. Agr. Pl. Dis. Rptr. 39: 20-22.

the tests here described, however, the reduction in some seed lots was sufficient to be serious unless offset by a higher rate of seeding. It is suggested that germination tests be made before planting soaked seed so that planting rates can be increased if germination is low.—Department of Plant Pathology, University of Wisconsin, Madison.

A Method for Testing Resistance of Beans to Bacterial Blights.[1] M. L. SCHUSTER.[2] In the course of genetic studies and breeding for resistance to halo blight (caused by *Pseudomonas phaseolicola* (Burk.) Dowson) and to common blight (caused by *Xanthomonas phaseoli* (E.F.Sm.) Dowson), it was found that water-soaking of bean leaves by close atomizing in the greenhouse or pressure spraying in the field enabled the bacteria to enter the leaves and cause infection.

The principle involved in this technique is the forcing of inoculum through the stomata into the leaves. The procedure for beans is to hold the leaf in the palm of the hand and to force the bacterial suspension through the stomata of the lower side. A paint sprayer held about 2 in. from the leaf surface proved satisfactory for water-soaking the leaves. Similar results were obtained in the greenhouse with a DeVilbiss No. 127 atomizer attached to an air line at 15 lb. pressure. In the field a power sprayer regulated at about 150 lb. pressure proved satisfactory for water-soaking the leaves. The nozzle was adjusted to release a stream of water unbroken until within a few in. of the plants where it separated into large droplets comparable to a rainstorm. This technique, initiated by Clayton[3] for inducing infection with bacteria causing tobacco wildfire, has since been employed by other workers.[4,5] Recently, Weindling[6] substantiated the usefulness of the technique in work on bacterial blight of cotton.

Inoculation of beans for halo blight by atomizing,[7] without water-soaking, has been reported[8] to have been most successful at the time the third trifoliate was unfolding. Infection with this inoculation technique was variable and slight on the primary leaves. In contrast, the water-soaking method has resulted in good infection of the primary leaves of susceptible varieties. The primary leaves of resistant varieties were resistant to infection, however, in both cases.

When primary leaves are used as indicators of halo blight reaction, plants can be inoculated about 14 days sooner than when later developing leaves are employed. Inoculation of 1 leaf per plant usually proved sufficient to obtain a disease reading. Since each plant is inoculated individually, disease escapes are less probable than following the usual methods of inoculation. Trifoliates on plants on which the reaction of primary leaves is questionable can be inoculated to confirm the first reading.

Beans were inoculated by water-soaking leaves of different aged plants at 3 temperatures to determine possible differences due to inoculation technique. The temperatures included 16, 21, and 27°C. The different ages of bean plants tested were 5, 6, 7, 8, and 9 days after planting. These were grown at 21° until the time of inoculation. In no case were any of the trifoliate leaves expanded at the time of inoculation. In this particular experiment an area of 8 × 11 mm on the primary leaf was inoculated with a standardized suspension of approximately 8 million bacteria per ml.

It is not uncommon for hydrosis or translucence and halo production to appear in 2 days after spraying, especially in the youngest plants. It is more satisfactory, however, to take readings 10–14 days after inoculation. The 21° temperature proved optimum for symptom expression although infection was noted on the susceptible varieties, Red Kidney and Asgrow Stringless, at 16° on plants of all ages tested. The production of halos around the infected areas of leaves of susceptible varieties was faint and limited at the lower temperature with hydrosis present in the lesion. At 27° the 5- and 6-day-old plants showed slight hydrosis and faint halo production around the periphery of the infected area. The variety Mexican Red was resistant at the 3 temperatures.

Age of the primary leaf at the time of inoculation has a definite effect on the size of halos produced. Infection of the younger leaves usually results in larger halos and more often in the development of systemic symptoms. Similarly, hydrosis at the site of inoculation is more pronounced on the primary leaves of younger plants.

A comparison of the disease reaction of primary leaves and trifoliates of the same plant was determined

[1] Published with the approval of the Director, Paper No. 693, Journal Series, Nebraska Agricultural Experiment Station.

[2] Associate Plant Pathologist, Department of Plant Pathology, Nebraska Agricultural Experiment Station, Lincoln, Nebraska.

The writer is grateful to Dr. W. B. Allington, Chairman, Department of Plant Pathology, for suggestions during the course of this study.

[3] Clayton, E. E. 1936. Water-soaking of leaves in relation to development of wildfire disease of tobacco. Jour. Agr. Res. 52: 239-269.

[4] Allington, W. B., and C. V. Feaster. 1946. The relation of stomatal behavior at the time of inoculation to the severity of infection of soybeans by Xanthomonas phaseoli var. sojense (Hedges) (Starr) Burk. Phytopathology 36: 385-386.

[5] Diachun, S., W. D. Valleau, and E. M. Johnson. 1942. Relation of moisture to invasion of tobacco leaves by Bacterium tabacum and Bacterium angulatum. Phytopathology. 32: 379-387.

[6] Weindling, R. 1948. Bacterial blight of cotton under conditions of artificial inoculation. U. S. Dept. Agr. Tech. Bul. 956, 59 p.

[7] General atomizing refers to atomizing without water-soaking, in contrast to close atomizing which refers to atomizing with water-soaking.

[8] Goss, R. W. 1940. The relation of temperature to common and halo blight in beans. Phytopathology 30: 258-264.

by inoculating them by close atomizing when they were about half expanded (Fig. 1). The primary leaf and the third trifoliate reacted similarly to halo blight on each of the varieties Asgrow Stringless, Mexican Red, Arikara Yellow, Giant Stringless, U. S. No. 5 Refugee, and Red Kidney.

To further test the value of the technique, more divergent germ plasm was employed using the F_2 generations of 3 different crosses and their reciprocals. In a previous paper, the author reported on genetic studies of halo blight in which the data were obtained from plants inoculated by the general atomizing method.[9] A total of 1786 of the same F_2 progeny gave fairly comparable results insofar as observed segregation was concerned when tested by the close-atomizing procedure.

Satisfactory results were also obtained for common blight using the susceptible common bean varieties Great Northern, Red Kidney, Scotts Bluff Pinto, and a resistant strain of tepary bean. The infected areas of the susceptible varieties exhibited hydrosis and in the periphery of these areas a yellow border appeared in about 7 days. In the inoculated areas of the resistant variety, small dry spots resulted with hydrosis absent.

The water-soaking technique is advantageous in that it can be used satisfactorily either in the greenhouse or field. In both cases, a satisfactory disease reaction can be obtained. A single inoculation of the primary leaves is usually sufficient for halo and common blight, providing adequate water-soaking is obtained at the time of inoculation. Time and labor is decreased since large plant populations can be tested in a relatively short time.—Department of Plant Pathology, Nebraska Agricultural Experiment Station, Lincoln, Nebraska.

[9] Schuster, M. L. 1950. A genetic study of halo blight reaction in Phaseolus vulgaris. Phytopathology 40: 604-612.

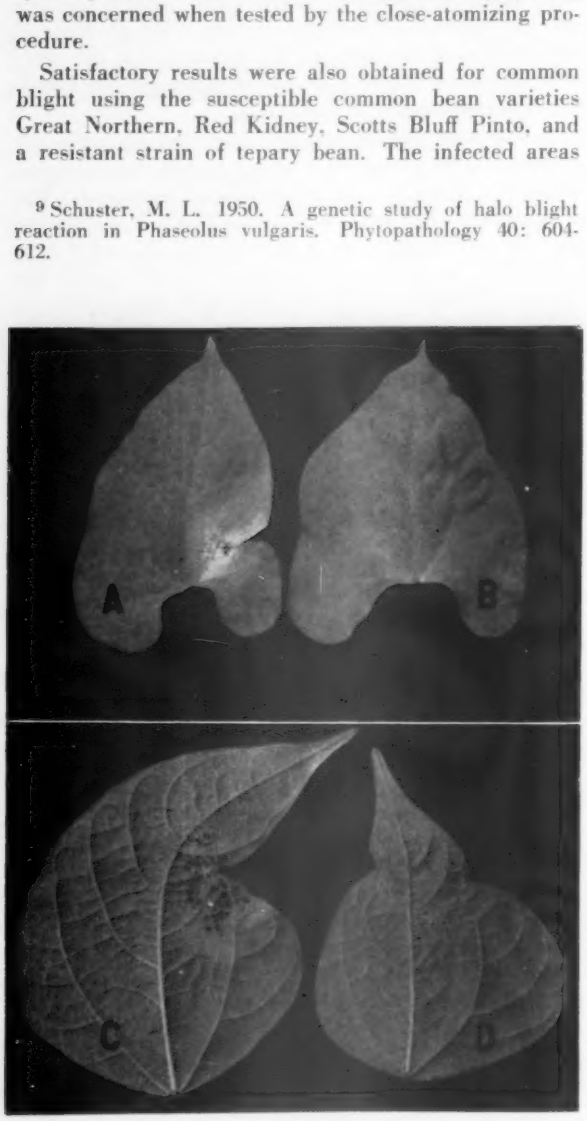

FIG. 1. Halo blight symptoms of beans following water-soaking method of inoculation. A) Primary leaf of susceptible variety. B) Primary leaf of resistant variety. C) Leaflet of susceptible variety. D) Leaflet of resistant variety.

Methyl Bromide Gas Treatments of Dormant Gladiolus Corms and of Sclerotia of Sclerotium rolfsii. T. R. CARPENTER AND EARLE T. GAMMON.[1] In January, 1954, the Bureaus of Plant Pathology and Entomology, California Department of Agriculture, conducted tests designed to ascertain the tolerances of dormant gladiolus corms and of mature viable sclerotia of *Sclerotium rolfsii* Sacc. to methyl bromide (bromomethane) gas. These tests were made to determine whether or not methyl bromide could be used in eliminating *S. rolfsii* from gladiolus corms grown in a commercial planting where this fungus was found to be present. Corms and sclerotia were treated simultaneously with methyl bromide gas at rates of 3, 5, 7.5, 10, 15, and 20 lb. per 1000 cu. ft. of air space in the treatment chamber. Each treatment was conducted at 27°C and relative humidity of approximately 60 per cent for 4 hours. About 15 days prior to the gas treatments, the corms had been treated for 30 minutes in a 1:1000 aqueous solution of mercuric chloride. The sclerotia, including checks, were treated in a 1:1000 aqueous solution of mercuric chloride for 30 seconds then dried before preparation for the gas treatments.

Dormant corms, 1200 each of the varieties Burma, Cordova, Florence Nightingale, and Snow Princess, were furnished by a commercial nursery. At each gas concentration treatment, 100 corms of each variety were treated and 100 were set aside as untreated checks. Following each treatment, the excess gas was allowed to escape from the corms by airing. The corms were planted by the nurseryman within 21 days after treatments.

In November, 1954, the nurseryman reported the following results of the gas treatments given the corms. No apparent differences were observed in emergence, growth, or production between the checks and the corms treated at gas concentration of 3 lb. per 1000 cu. ft. From 0 to 10 per cent emergence was noted

[1] Respectively Associate Plant Pathologist, Bureau of Plant Pathology, and Economic Entomologist, Bureau of Entomology, California Department of Agriculture, Sacramento.

for the corms that had been treated with 5, 7.5, and 10 lb. per 1000 cu. ft. A varietal difference also was reported. A stimulation was noted for these emerged plants. No corms grew which had been treated with 15 or 20 lb. per 1000 cu. ft., and partial to total breakdown of them was noted at planting time.

In the preparation of the sclerotia of *S. rolfsii* for each gas-concentration treatment and for checks, 20 sclerotia in sterile Petri plates, 1 plate per preparation, were used for each of the following preparations: 1) dry sclerotia; 2) sclerotia maintained moist on filter paper saturated with distilled water; 3) sclerotia seeded on potato-dextrose agar, pH 5.6; 4) same as preparation 3; and 5) untreated sclerotia. The Petri plates that were gas treated were uncovered during treatment and for a short time immediately afterward in order to allow the gas to escape. All preparations were replicated 4 times. Sclerotia from preparations 1, 2, 3, and 5 were immediately transferred to fresh potato-dextrose agar in Petri plates and incubated for 5 days at 30°C, then held at room temperature for an additional 15 days. Sclerotia from preparations 4 were incubated for 5 days at 30° without removal from the media on which they were gas treated; after the 5-day incubation, approximately ½ of the sclerotia from each of the 4 replications were transferred to fresh media in order to test the influence of the treated media.

Of the 480 sclerotia used as checks, 478 were viable. The sclerotia exposed to the gas in a dry condition at all gas concentrations (preparations 1) were not significantly different from the checks; 464 of the 480 treated proved viable. The sclerotia exposed to the gas on water-saturated filter paper (preparations 2) were not significantly different from the checks for the gas concentration treatments of 3, 5, 7.5, and 10 lb. per 1000 cu. ft.; however, only 34 of the 80 sclerotia treated at the 15-lb. rate survived, which was significantly different from the checks, and no sclerotia of this preparation survived the 20-lb. treatment. The sclerotia exposed to the gas treatments on potato-dextrose agar and then transferred to fresh media for incubation (preparations 3) were not significantly different from the checks for treatments of 3, 5, 7.5 and 10 lb. per 1000 cu. ft.; no sclerotia of this preparation survived the 15- or 20-lb. gas treatments. No sclerotia of preparation 4, exposed to the gas treatments on potato-dextrose agar and incubated on the same media, survived any gas treatment; viability could not be demonstrated in those that were transferred to fresh media following the 5-day incubation. Preparation 4, however, is not applicable to practical methods of treating corms.

These tests demonstrate that dormant gladiolus corms will not tolerate treatments with methyl bromide gas at concentrations necessary to kill sclerotia of *S. rolfsii*.—Bureau of Plant Pathology and Bureau of Entomology, California Department of Agriculture, Sacramento, California.

A Haplo-lethal Deficiency in Ustilago kolleri.[1] VINCENZO GRASSO.[2] Many workers have dealt with genetics and sex-compatibility factors of the smut fungi of oats (*Ustilago avenae* (Pers.) Rostr. and *U. kolleri* Wille), and all of them report that these species possess 2 sex groups.[3] This paper is a report of a sex-related phenomenon observed for the first time in a collection of *U. kolleri* from Parma, Italy.

The 4 sporidia (designated a, b, c, and d in the order of isolation) were isolated from the promycelium of several germinating chlamydospores and cultured on potato-dextrose agar (Difco). Two of the sporidia from each chlamydospore multiplied rapidly to produce normal monosporidial lines, whereas the other 2 budded a few times and then ceased to grow (Fig. 1). Furthermore, no fusions occurred when sporidia from colonies initiated by the 2 rapidly multiplying sporidia were mixed on water agar, whether the lines originated from the same or from different chlamydospores (Table 1). On the other hand, when sporidia from colonies initiated by the 2 rapidly multiplying sporidia were mixed with complete sets of 4 lines from other collections (3 from Italy and 1 from the United States), they fused with sporidia of 1 sex-compati-

[1] This study was supported by research grants from Consiglio Nazionale delle Ricerche (Roma) and from Exchange Visitor Program No. G-5 (Public Law 584) 79th Congress, U.S.A. The writer is indebted to Drs. E. C. Stakman, J. J. Christensen, and J. B. Rowell of the University of Minnesota for their assistance in selecting this problem and in initiating the investigation. Special gratitude and indebtedness are expressed to Drs. C. H. Holton and G. W. Fischer of the U. S. Department of Agriculture and Washington State College, respectively, for their instructions on techniques and suggestions on interpretation of results.

[2] Professor of Plant Pathology, University of Florence, Italy.

[3] Holton, C. S. 1953. Physiologic specialization and genetics of the smut fungi. II. Bot. Rev. 19: 187-208.

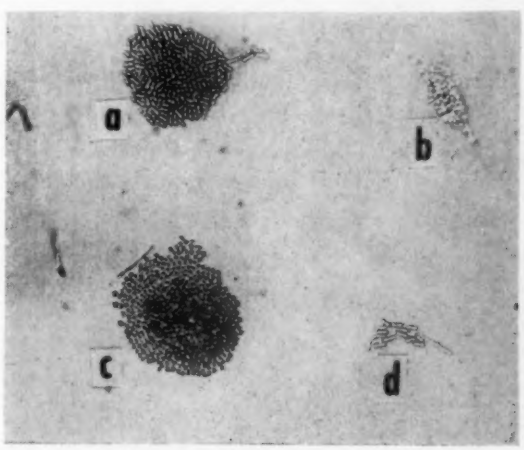

FIG. 1. Photomicrograph showing colony development of 4 sporidia isolated from the promycelium of 1 germinating spore of *Ustilago kolleri* (collection from Parma). a, c) Normal colony development. b, d) Arrested colony development in haplo-lethal deficient lines.

TABLE 1.—*Compatibility reactions*[a] *of 6 monosporidial isolates from 4 chlamydospores of U. kolleri (collection from Parma)*

Line[b]	C-a	C-b	A-c	A-d	H-a	I-c
C-a	—	—	—	—	—	—
C-b	—	—	—	—	—	—
A-c	—	—	—	—	—	—
A-d	—	—	—	—	—	—
H-a	—	—	—	—	—	—
I-c	—	—	—	—	—	—

[a] — indicates no fusion.
[b] Large letters designate chlamydospores; small letters designate monosporidial lines from each spore.

TABLE 2.—*Compatibility reactions*[a] *of 2 normal monosporidial lines (a and b) from chlamydospore E of the Parma collection mated with certain monosporidial lines from each of 3 other collections*

Collection and line[b]	Parma		Windom		Siracusa		Firenze	
	E-a	E-b	D-a	D-c	C-a	C-b	E-a	G-a
Parma E-a	—	—	—	+	+	—	+	—
Parma E-b	—	—	—	+	+	—	+	—
Windom D-a	—	—	—	+	+	—	+	—
Windom D-c	+	+	+	—	—	+	—	+
Siracusa C-a	+	+	+	—	—	+	—	+
Siracusa C-b	—	—	—	+	+	—	+	—
Firenze E-a	+	+	+	—	—	+	—	+
Firenze G-a	—	—	—	+	+	—	+	—

[a] + indicates fusion; — indicates no fusion.
[b] The Windom collection was from Minnesota, U.S.A., and the others from Italy. Large letters designate chlamydospores, and small letters designate monosporidial lines from each spore.

bility group in each set (Table 2). It was evident from these results that the sporidia that multiplied rapidly to produce monosporidial lines of the Parma collection were of 1 sex group only. The lethal factor that was observed in the sporidia that failed to grow beyond a few buddings seemed to be closely linked with the other sex group in *U. kolleri.*

The failure of 2 sporidia to grow is not a new phenomenon in the smut fungi, nor is it common. Cases of sex-linked haplo-lethal deficiencies have been reported in *Ustilago bullata* Berk.[4] and *Tilletia foetida* (Wallr.) Liro.[5] The same phenomenon occurs in *U. kolleri* as judged by the behavior of the Parma collection, i.e. the failure of 2 of the sporidia to grow, and by the mating tests reported. This sex-linked haplo-lethal deficiency is not like the cases of lethal deficiency reported for *Ustilago zeae*[6] and for *Sphacelotheca sorghi,*[7] which were related to abnormal chlamydospore germination followed by promycelial degeneration.

The results in Table 1 might suggest that the Parma collection cannot produce infection. This is not the case, however, since this collection reproduces itself when a susceptible variety of oats is inoculated with chlamydospores. Furthermore, when masses of chlamydospores are germinated on an agar medium, many aerial hyphae develop. These hyphae resemble the aerial dikaryotic hyphae produced on agar when compatible normal monosporidial lines are combined, but it has not yet been determined whether these aerial hyphae originate from fused sporidia, fused promycelial cells, or fusions between sporidia and promycelial cells.—University of Minnesota, St. Paul, and State College of Washington, Pullman.

[4] Fischer, G. W. 1940. Two cases of haplo-lethal deficiency in Ustilago bullata operative against saprophytism. Mycologia 32: 275-289.

[5] Holton, C. S. 1951. Method and results of studies on heterothallism and hybridization in Tilletia caries and T. foetida. Phytopathology 41: 511-521.

[6] Chilton, St. J. P. 1938. The occurrence of lysis in certain crosses of Ustilago zeae. (Abs.) Phytopathology 28: 5.

[7] Laskaris, T. 1939. The occurrence of lysis in certain crosses of Sphacelotheca sorghi. (Abs.) Phytopathology 29: 14.

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- is a very useful aid in the identification of newly isolated strains of the Gram-negative intestinal bacteria. Exceptionally brilliant aerobic and anaerobic fermentation reactions are produced in tubes of this medium. The indicator used is Phenol Red.

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DETROIT 1, MICHIGAN

Acting Head, is the new title for M. B. Linn who assumed his administrative duties July 1 in the newly created Department of Plant Pathology, Illinois College of Agriculture and Agricultural Experiment Station. In addition to his administrative duties, Dr. Linn will continue his projects on vegetable disease investigations and teaching courses in the Department.

New Quarters were obtained by the Department of Botany and Plant Pathology, Purdue University, when it moved to the new Life Science Building this spring. Phytopathologists who have occasion to travel in the vicinity of Lafayette, Indiana, are cordially invited to visit the Department and inspect its air-conditioned laboratories, conveniently located greenhouses, disease resistance work rooms and controlled climate units.

Do not forget to make your reservations for the Atlanta meeting through the AAAS Housing Bureau at the earliest possible date. Headquarters for the Society will be in the Atlanta Biltmore Hotel.

High honor was accorded J. C. Walker when he was presented with a bronze plaque at the annual convention of the American Seed Trade Association. The plaque carried the inscription: "Dr. John Charles Walker: Scientist, Plant Pathologist, Inspirational Teacher, Author; internationally eminent in the field of research and distinguished for his practical scientific knowledge applied to breeding disease-resistant vegetables for the benefit of mankind."

Phytopathologist E. C. Stakman will deliver one of three general lectures at the 14th International Horticultural Congress to be held August 29 to September 6 at Scheveningen, Holland. His topic will be "The Application of Atomic Energy in Horticulture."

A Distinguished Service Award was recently presented by the U. S. Department of Agriculture to Society member Gotthold Steiner. Dr. Steiner was honored for outstanding leadership in conducting research on nematodes and their control, emphasizing their importance in limiting crop production and for advancing the science of nematology on an international level.

After 36 years' service C. W. Hungerford, Professor and Head of the Department of Plant Pathology, University of Idaho has retired from his duties. Dr. Hungerford spent most of his 36 years working on bean diseases and bean improvement. In addition to his duties as Head of the Department, Dr. Hungerford served at various periods as Assistant Dean, College of Agriculture; Vice-Director of the Experiment Station; and Dean of the Graduate School.

After 33 years' service W. B. Tisdale retired August 1 as Head of the Department of Plant Pathology, University of Florida. Dr. Tisdale is widely known for his research on black shank and other diseases of tobacco. He will continue to reside in Gainesville.

Editor: K. W. KREITLOW, Plant Industry Station, Beltsville, Md.

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